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Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.01

Topic: B.11. Glial Mechanisms

Support: NINDS Grant - NS104160
NIA ADC Grant - AG013854

Title: Differential Phagocytosis of Fibrillar and Soluble Oligomeric Aβ by Primary Human Microglia in Culture

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Abstract: The amyloid-β (Aβ) peptide accumulates in plaques in the brains of patients with Alzheimer's disease (AD) and contributes to disease pathogenesis. Aβ forms aggregates that assume various conformations based on size and arrangement of oligomers. Large aggregates of Aβ can form fibrils, with abnormal β-pleated sheet conformation, that deposit in compact, neuritic plaques, and have been shown to exert toxic effects on neurons. Smaller soluble Aβ oligomers disperse across various brain compartments and are proposed to cause synaptotoxicity. One mechanism of Aβ clearance from the brain involves phagocytosis and degradation by microglia, the resident macrophages of the central nervous system. While it is known that microglia phagocytose Aβ, it is not known whether they phagocytose fibrillar and soluble oligomeric Aβ with the same level of efficiency. Furthermore, the ability of cultured primary human microglia to phagocytose each Aβ conformation has not been investigated. In this study we investigated phagocytosis of fibrillar and soluble oligomeric Aβ by cultured primary human microglia. Microglia were isolated and cultured from postmortem human brains using protocols in routine use at our laboratory. Cells were seeded in multi-chamber slides and treated with 0, 2.5, 5 and 10 µg/100µL of fibrillar or soluble oligomeric Aβ conjugated to the 5-FAM fluorescent tag suspended in sterile BSA/RPMI (1mg/mL). The optical density of 5-FAM fluorescence in individual microglia was determined in triplicate experiments as a measure of Aβ phagocytosis. Both fibrillar and soluble oligomeric Aβ were phagocytosed by microglia in a dose-dependent manner. However, soluble Aβ oligomers were phagocytosed more efficiently than fibrillar Aβ. At every dose, optical density of 5-FAM soluble oligomeric Aβ was significantly greater than the control condition ($p<0.001$), while this difference reached significance only at the highest dose of fibrillar Aβ ($p<0.001$). Furthermore, at 5 and 10 µg/100µL concentrations, optical density of 5-FAM soluble Aβ oligomers were significantly

greater than fibrillar Aβ. 5-FAM fibrillar Aβ was often observed in clumps extracellularly, and apposed to microglia membranes. These findings indicate that human microglia phagocytose soluble Aβ oligomers much more readily when compared with fibrillar Aβ. Continuous interaction of extracellularly accumulating fibrillar Aβ with receptors on microglia membranes is likely to contribute to the inflammatory activation of microglia observed in AD.

Disclosures: A. Bahrami: None. **C. Swope:** None. **E. Taefi:** None. **M. Flanagan:** None. **T. Gefen:** None. **M. Mesulam:** None. **C. Geula:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.02

Topic: B.11. Glial Mechanisms

Title: Strategies for studying the role of TREM2 in microglial phagocytosis using iPSC-derived "brain-in-a-dish" cell models

Authors: C. MUNN, M. K. LIVINGSTON, M. E. GOEDLAND, S. BURTON, S. HILCOVE, *C. B. CARLSON, W. W. POON, D. RAJESH;
FUJIFILM CDI, Madison, WI

Abstract: Microglia are immune cells that ordinarily maintain homeostasis in the brain; however, microglial dysfunction has been implicated in many neurodegenerative disorders, including Alzheimer's Disease (AD). Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) has been identified as a novel potential therapeutic target for AD as it has been reported to regulate and alter the phagocytic function of microglia. Due to the limited availability of primary microglia, human induced pluripotent stem cell (iPSC)-derived microglia fill the critical gap for large-scale drug discovery applications. The present study focuses on the quantification of functional differences associated with TREM2 expression in an isogenic cellular system to identify the key regulators of phagocytic function of microglia. Cryopreserved iPSC-derived microglia from either an apparently healthy normal (AHN) donor or isogenic, gene-edited TREM2 knockout (KO) lines [both heterozygous (HZ) and homozygous (HO)] revealed altered kinetics of phagocytosis with bacterial particles and fibrillated amyloid beta. All three microglia lines were used to screen 32 different media conditions which identified two essential growth factors required for sustaining survival and amyloid beta phagocytosis. When grown in tri-culture with AHN iPSC-derived astrocytes and iPSC-derived neurons (both GABAergic and glutamatergic), microglia exhibited enhanced phagocytosis in tri-culture compared to mono-culture. These functional studies also illustrated that the consequences of TREM2 expression on phagocytic activity were dependent on the type of substrate, its concentration, and the media conditions used in the assay. These data underscore the power of

using iPSC-derived microglia to explore the role of TREM2 in neurodegenerative diseases and highlight the range of phenotypes observed with such “brain-in-a-dish” cell models.

Disclosures: **C. Munn:** A. Employment/Salary (full or part-time):; FCDI. **M.K. Livingston:** A. Employment/Salary (full or part-time):; FCDI. **M.E. Goedland:** A. Employment/Salary (full or part-time):; FCDI. **S. Burton:** A. Employment/Salary (full or part-time):; FCDI. **S. Hilcove:** A. Employment/Salary (full or part-time):; FCDI. **C.B. Carlson:** A. Employment/Salary (full or part-time):; FCDI. **W.W. Poon:** A. Employment/Salary (full or part-time):; FCDI. **D. Rajesh:** A. Employment/Salary (full or part-time):; FCDI.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.03

Topic: B.11. Glial Mechanisms

Support: CIHR Foundation Grant 353750

Title: The dark microglial subset displays ultrastructural and metabolic alterations in an aged mouse model of beta-amyloid pathology

Authors: *M.-K. ST-PIERRE[1], M. CARRIER[1], F. GONZALEZ IBANEZ[1], E. SIMONCICOVA[2], M.-J. WALLMAN[3], L. VALLIÈRES[4], M. PARENT[3], M.-E. TREMBLAY[2]; [1]Univ. Laval, Quebec, QC, Canada; [2]Div. of Med. sciences, Univ. of Victoria, Victoria, BC, Canada; [3]Psychiatry and Neurosci., [4]Neurosciences, Laval Univ., Quebec, QC, Canada

Abstract: Recent technical advances helped to reveal the considerable heterogeneity of microglia, the resident immune cells of the central nervous system. One of these microglial subsets, the dark microglia (DM), was characterized by ultrastructural signs of cellular stress such as dilated endoplasmic reticulum (ER), altered mitochondria and loss of heterochromatin pattern. These dark cells were previously identified in middle-age wild-type (WT) controls as well as near amyloid beta plaques in age-matched APP-PS1 mice, a mouse model of amyloid beta deposition. However, their ultrastructural features and interactions with hallmarks of amyloid deposition (plaques, dystrophic neurites) were not yet investigated in aging. Using high-magnification chip mapping by scanning electron microscopy, we first analyzed the density of both typical and DM in the dorsal hippocampus CA1 (strata *lacunosum-moleculare* and *radiatum*) of 20-month-old male WT *versus* APP-PS1 mice. The density of DM per mm^2 profoundly increased in the APP-PS1 mice compared to WT controls, comprising nearly 20% of all microglial cells present in the *stratum lacunosum-moleculare*. We found DM interacting more with dystrophic neurites, while contacting less healthy synaptic elements, myelinated axons and brain vasculature compared to typical microglia. Moreover, DM possessed more dilated ER and

Golgi apparatus, as well as more mitochondrial alterations, alongside a cytoplasmic accumulation of glycogen granules. The present study further highlights the close interactions between DM and amyloid beta deposition hallmarks, suggesting a specialized involvement, whether beneficial or detrimental, compared to typical microglia in this context. Further investigations into the prevalence and parenchymal interactions of DM with Alzheimer's disease hallmarks will be performed in *post-mortem* brain samples from Alzheimer's patients and aged-matched controls. Studying DM close interactions with disease hallmarks could help us find new therapeutic targets modulating these cells in a context-dependent manner.

Disclosures: M. St-Pierre: None. **M. Carrier:** None. **F. Gonzalez Ibanez:** None. **E. Simoncicova:** None. **M. Wallman:** None. **L. Vallières:** None. **M. Parent:** None. **M. Tremblay:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.04

Topic: B.11. Glial Mechanisms

Support: USUHS Program Project
CHIRP (NHLBI/NIH and USUHS)
DoD for CNRM

Title: Interaction of microglia and vasculature in mouse model of chronic high altitude adaptation

Authors: *K. WHITING[1], T. BARVIR[2], X. XU[2], J. BARNIE[3], S. JAISWAL[4], F. LISCHKA[5], A. KOROTCOV[4], A. KNUTSEN[4], C. WANG[8], M. WANG[8], N. P. CRAMER[2], V. TSYTSAREV[2], G. YU[8], J. J. ILIFF[9], C. BROWNE[6], B. J. DARDZINSKI[10], D. L. DICKSTEIN[7], D. P. PERL[7], Z. GALDZICKI[2];
[1]Grad. Program in Neurosci., [2]Dept. of Anatomy, Physiol. and Genet., [3]Comprehensive Student Res. Training Program, [4]Dept. of Radiology and Radiological Sci., [5]Biomed. Instrumentation Ctr., [6]Dept. of Pharmacol., [7]Dept. of Pathology, Uniformed Services Univ., Bethesda, MD; [8]Bradley Dept. of Electrical & Computer Engin., Virginia Tech., Arlington, VA; [9]VA Puget Sound Hlth. Care Syst., Univ. of Washington Sch. of Med., Seattle, WA; [10]NIH, Bethesda, MD

Abstract: Exposure to hypobaric hypoxia during extended stays at high altitude (HA) is known to cause maladaptive changes to brain physiology and function. HA compromises tissue oxygenation, which has serious implications for overall health and cognitive performance often lasting long after return to normoxic conditions. Previously, our mouse models have revealed enhanced angiogenesis and BBB leakage, increased microglial phagocytosis, and deficits in

hippocampal mediated behavior following HA. In our model, HA exposure is achieved by placing ~7 week old mice in a hypobaric chamber to a simulated altitude of 5000 m (10-11% O2, ~60% SpO2), with chronic exposures ranging 3-12 weeks. Here we assess the role of inflammation and neurovascular adaptive responses to HA. We identify significant increases in neurovascular structure through microCT imaging, with changes in average vessel diameter and tortuosity, along with region specific changes in BBB dysfunction. We also measured distinct regional differences in microglia movement dynamics through ex vivo 2-photon imaging of Cx3Cr1-GFP mice, showing an increase in process tip speed in response to laser ablation of microvasculature. These changes may be associated with variations in inflammatory cytokines (IL-6, TNFα) and increased expression of SDF-1α (involved in angiogenesis and microglia chemotaxis) in peripheral blood serum and brain homogenate samples. Increased levels of AQP-4 and cerebral arteriole pulsatility after HA corresponds to our EEG findings of decreased slow wave sleep and increased time awake, suggesting an impact of HA on glymphatics function. In summary, HA exposure significantly impacts neurovasculature and microglia interactions, leading to cognitive impairment with possible effects on glymphatic function. This is a serious health concern for those living and working at HA, and a potential risk factor for the development of neurodegenerative diseases.

Disclosures: K. Whiting: None. **T. Barvir:** None. **X. Xu:** None. **J. Barnie:** None. **S. Jaiswal:** None. **F. Lischka:** None. **A. Korotcov:** None. **A. Knutsen:** None. **C. Wang:** None. **M. Wang:** None. **N.P. Cramer:** None. **V. Tsytsarev:** None. **G. Yu:** None. **J.J. Iliff:** None. **C. Browne:** None. **B.J. Dardzinski:** None. **D.L. Dickstein:** None. **D.P. Perl:** None. **Z. Galdzicki:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.05

Topic: B.11. Glial Mechanisms

Support: NIH/NIGMS Grant GM122657

Title: Sensory deprivation impacts microglia morphology and perineuronal net integrity in a manner that varies from that of chronic LPS challenge in early development

Authors: *A. BARRIENTOS[1], S. MROZIUK[2], A. LAHIJANI[3], J. C. BRUMBERG[2]; [1]Psychology, CUNY The Grad. Ctr., New York, NY; [2]Psychology, CUNY Queens Col., Flushing, NY; [3]New York Inst. of Technol., Old Westbury, NY

Abstract: Microglia are an integral part of the cellular constituents of the central nervous system with an indispensable role in the development and refinement of neuronal circuits during development. To date, limited studies focused on how microglia interact with the perineuronal

net (PNN), a neuron-associated form of the extracellular matrix in the developing brain. Using the barrel cortex as a model system, we examined how peripheral manipulations through chronic lipopolysaccharide (LPS) or minocycline administration alone or in conjunction with sensory deprivation impact microglia in the primary somatosensory cortex (S1). We also examined how microglia's reactive state as characterized by their morphology correlate to changes to the PNN in S1. C57BL/6 mouse litters were randomly assigned to be given intraperitoneal injections of saline, minocycline (a microglia inhibitor) or LPS (an inflammatory agent). A separate cohort of litters underwent sensory deprivation through whisker trimming in addition to receiving saline, minocycline or LPS. Pups received their assigned treatment from post-natal day 1 (P1) until P30. Microglia morphology, density and PNN density and integrity were examined using immunohistochemical (IBA1 was used to visualize microglia) and histochemical staining (WFA was used to visualize PNNs) and stereology. We tested the following hypotheses: (1) trimming coupled with LPS injection will interact synergistically resulting in fewer PNNs, and (2) minocycline will prevent microglia from becoming reactive and little to no impact will be observed to the PNN. We found that microglia morphology is impacted in a treatment-specific manner. Minocycline results in hyper ramification of microglial processes, while LPS resulted in smaller microglia somas and retracted processes. Sensory deprivation tapers these effects. LPS in conjunction with sensory deprivation results in a reduction of PNN density and diminished staining intensity. Female mice had fewer PNNs in S1 following sensory deprivation relative to males. Sensory deprived mice had reduced surface area of WFA+ somas relative to controls and those of minocycline and LPS-treated mice. A regression analysis shows that microglia density as well as specific morphological features correlated with fewer PNNs following sensory deprivation. Both minocycline and LPS treatment disrupt this relationship. Taken together, these studies suggest that sensory deprivation may render the developing brain more vulnerable to factors that influence microglia activity which may be related to PNN maturation.

Disclosures: A. Barrientos: None. **S. Mroziuk:** None. **A. Lahijani:** None. **J.C. Brumberg:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.06

Topic: B.11. Glial Mechanisms

Support: NIH AG058068
Cure Alzheimer's Fund

Title: Apoe genotype and sex modulate lipid droplet accumulation in microglia

Authors: *T. IIDA, C. J. PIKE;
Leonard Davis Sch. of Gerontology, USC, Los Angeles, CA

Abstract: *APOE* genotype and sex modulate lipid droplet accumulation in microglia
T. Iida, C.J. Pike
Microglia are key immunomodulators of Alzheimer's disease (AD) pathogenesis. Biological sex and apolipoprotein E (*APOE*) genotype are significant regulators of not only AD risk, but also microglial phenotypes including those implicated in AD. Sex and *APOE* genotype also affect lipid profile and metabolism. Indeed, as high plasma lipid levels are associated with AD progression, higher plasma neutral lipids in *APOE4* carriers may contribute to *APOE4*-associated AD risk. Recently, accumulation of neutral lipids in the form of lipid droplets (LDs) has been observed within microglia in aging and AD brains and associated with impaired microglial function and altered transcriptomic profile. These findings suggest the modulation of microglial lipid metabolism may be highly relevant to understanding AD pathogenesis and developing interventions. Unknown is how *APOE* genotype and biological sex affect LD accumulation in microglia and the functional consequences of these relationships. To investigate these issues, we utilized primary microglia cultures generated from young adult (age 3-4 months) male and female mice homozygous for knock-in of human *APOE3* or *APOE4*. Since lipid metabolism varies among different sex and *APOE* genotypes, first we examined LD accumulation using the neutral lipid stain BODIPY under basal conditions and following stimulation with palmitic acid (PA) or lipopolysaccharide (LPS). Next, because LDs act as a form of energy storage and can have a protective role during cellular stress, we measured LD content and cell survival under starving stress conditions. Third, as lipid metabolism is strongly correlated with amyloid β (Aβ) clearance, we evaluated the relationship between LD content and clearance of oligomeric A β under basal conditions and following stimulation with PA or LPS. A range of individual and interactive outcomes were obtained across *APOE* genotypes and sex with a common theme of increased LD levels and poorer functional outcomes with *APOE4* and female sex. These findings provide new insight into the roles of *APOE* genotype and biological sex in AD risk with respect to regulation of microglial lipid metabolism and function.

Disclosures: T. Iida: None. **C.J. Pike:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.07

Topic: B.11. Glial Mechanisms

Support: R01AG062716
Faculty Start-Up Funds to LKF

Title: Estrogen deficiency in mice sensitizes hippocampal microglia to an immune challenge and alters behaviors

Authors: *K. SANCHEZ, S. L. WU, R. KAKKAR, J. S. DARLING, C. S. HARPER, R. CHEN, L. K. FONKEN;
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Abstract: Estrogen deficiency in women is linked to a heightened susceptibility to the development of mood disorders; indeed, oophorectomized women are 36% more likely to suffer from depression. Estrogens may exert neuroprotective effects by acting on microglia, the primary immune cell in the brain, and inhibiting neuroinflammation. Our objective was to determine the microglia-specific mechanisms through which estrogen deficiency alters neuroinflammatory responses, increasing susceptibility to behavioral deficits in a rodent model. C57BL/6 mice underwent either a bilateral ovariectomy or sham operation and received estradiol replacement or vehicle of sesame oil every four days to mimic their estrous cycle. After one month, mice were injected with saline or lipopolysaccharide (LPS) to stimulate an immune response. Our results indicate that ovariectomized mice tended to have greater anxiety-like behaviors and displayed exaggerated sickness anhedonia that was rescued by estradiol replacement. Iba1 immunoreactivity and microglia density in the hippocampus were elevated in estrogen-deficient mice; however, soma-specific features suggestive of microglia activation were not altered by estrogens. This was accompanied by exacerbated expression of the pro-inflammatory cytokine *Il6* after an immune challenge that was blocked by estradiol replacement. Moreover, *Cx3cr1* and *Trem2* (microglial receptors that maintain quiescence and limit activation) were downregulated at baseline in estrogen-deficient mice, indicating that estrogen loss may sensitize microglia to immune stimuli. Collectively, these results suggest that lack of estrogens may regulate microglia to confer exaggerated neuroimmune responses, thereby raising vulnerability to social- and anxiety-related behavioral changes. Since most women undergo permanent loss of ovarian function at some point in their lives, it is essential to uncover the neuroimmune mechanisms by which declining estrogen levels impact behavior.

Disclosures: K. Sanchez: None. **S.L. Wu:** None. **R. Kakkar:** None. **J.S. Darling:** None. **C.S. Harper:** None. **R. Chen:** None. **L.K. Fonken:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.08

Topic: B.11. Glial Mechanisms

Support: R01AA025591
R01AA016959

T32AA007471

Title: Microglial 3D reconstruction indicates subtle shifts in morphology following repeated binge ethanol exposure in female adolescent rats

Authors: *J. MELBOURNE[1], K. NIXON[2];
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Abstract: Adolescence is a critical developmental window during which time the brain is particularly sensitive to alcohol (EtOH) insult. EtOH exposure damages the adolescent brain and leads to activation of the neuroimmune system, including microglia. Microglia play critical roles in brain homeostasis, ongoing developmental processes such as synaptic refinement, myelination, and neurogenesis, as well as response to tissue injury. Microglia also exhibit innate immune memory such that ongoing functionality is impacted by prior stimuli, and our *ex vivo* studies indicate that ethanol induces a "primed" microglial state. While the literature suggests that females may be more sensitive to the neurotoxic effects of ethanol, most research has been carried out in males. Here, female adolescent rats (PND 30) were exposed to either a single or double ethanol binge, and alterations to microglial morphology were quantified. Rats in the single binge group (CE) were gavaged with isocaloric control diet every 8 h for 2 days (modified Majchrowicz model), allowed to recover for 7 days, and then gavaged with 25% (w/v) ethanol diet for a further 2 days. The double binge group (EE) received ethanol diet in both binges, and the remaining group control diet (CC), throughout. Tail bloods were collected immediately following each binge, with mean blood ethanol concentrations of 316.9 ± 13.0 mg/dl that did not differ significantly between groups. Following the last gavage rats were transcardially perfused with phosphate buffered saline and brains post-fixed in paraformaldehyde, sectioned at 4μm on a vibrating microtome, and fluorescence immunohistochemistry performed for microglia-specific marker, IBA1. Images from the dorsal dentate gyrus (molecular layer) and peri-/entorhinal cortices were acquired with a laser scanning confocal microscope (Olympus FV3000), and 3D reconstructed and analyzed using Imaris (Bitplane). Analyses included individual microglial volumetric and branching measurements. Results demonstrated an exposure-dependent decrease in microglial sphericity and a trend towards increase in total branch length and points with repeated ethanol exposure, though shifts in morphology were subtle. These results indicate subtle morphological changes to microglia following binge exposure in female adolescent rats, specifically hyper-ramification, and suggest a cumulative effect of repeated binge exposure consistent with priming. Alterations to microglial functionality may contribute to developmental perturbations following binge ethanol exposure in adolescents.

Disclosures: J. Melbourne: None. **K. Nixon:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.09

Topic: B.11. Glial Mechanisms

Title: High-resolution mass spectrometry analysis reveals novel histone modifications in microglia derived from a mouse model of chronic pain

Authors: P. ZHANG[1], J. GUERGUES[2], A. ALLEYNE[1], T. CIRINO[1], O. NADEAU[3], A. FIGUEROA[1], H. STACY[1], T. SUZUKI[4], J. MCLAUGHLIN[1], S. STEVENS, JR.[2], **B. LIU**[1]; [1]Univ. of Florida, Gainesville, FL; [2]Univ. of South Florida, Tampa, FL; [3]Albany Col. of Pharm. and Hlth. Sci., Colchester, VT; [4]Osaka Univ., Osaka, Japan

Abstract: Microglia play an important role in the maintenance of homeostasis in the CNS, and microglial dysregulation has been associated with the development and maintenance of chronic pain. However, the relevant molecular pathways remain poorly defined. In this study, we used a high-resolution mass spectrometry-based proteomic approach to globally profile histone protein modifications in microglia isolated from control and neuropathic pain mice. We identified several novel histone modifications including significantly decreased histone 3 lysine 27 mono-methylation and lysine 56 tri-methylation, as well as histone 3 tyrosine nitration associated with pain. Treatment of cultured microglia with an inhibitor of lysine demethylase 7A which is responsible for the demethylation of histone 3 lysine 27 mono-methylation significantly elevated its levels. Treatment of mice with neuropathic pain with the same inhibitor of lysine demethylase 7A markedly alleviated experimental pain in the mice. The identification of specific chromatin marks in microglia associated with chronic pain may yield critical insight into the contribution of glial cells to the development and maintenance of pain, and open new avenues for the development of novel therapeutics for effective management of chronic pain.

Disclosures: P. Zhang: None. **J. Guergues:** None. **A. Alleyne:** None. **T. Cirino:** None. **O. Nadeau:** None. **A. Figueroa:** None. **H. Stacy:** None. **T. Suzuki:** None. **J. McLaughlin:** None. **S. Stevens, Jr.:** None. **B. Liu:** None.

Poster

P163. Microglia: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P163.10

Topic: B.11. Glial Mechanisms

Support: F.90002539.02.004

Title: Therapeutic immune-modulation of human microglial cells using α-gal nanoparticles

Authors: *B. GOPALAKRISHNAN[1,2], U. GALILI[3], A. DUNBAR[2], L. SOLORIO[1], R. SHI[1,2,4], J. LI[4,2];
[1]Weldon Sch. of Biomed. Engineering-Purdue Univ., West Lafayette, IN; [2]Vet. Ctr. for Paralysis Research, Purdue Univ., West Lafayette, IN; [3]Dept. of Intrnl. Medicine, Rush Med. Sch., Chicago, IL; [4]Dept. of Basic Med. Sciences, Purdue Univ., West Lafayette, IN

Abstract: Spinal cord injury (SCI) is a devastating condition affecting 17,500 people per year with no FDA approved treatments. In the USA, nearly 253,000 people suffer from disabilities related to SCI, with annual medical costs exceeding $9.7 billion. SCI pathophysiology is a complex phenomenon and involves a multitude of orchestrated cellular players including blood derived macrophages and resident microglia. These immune cells serve multiple functions such as phagocytosis of cellular debris, inflammation involvement, generation of reactive oxygen/nitrogen species, and secretion of growth factors. It has been suggested that promoting the anti-inflammatory (pro-healing) macrophage/microglia phenotype within the post-injured microenvironment may be beneficial for mitigating tissue degeneration and improving functional recovery. Previous findings in wound healing research have shown that lipid nanoparticles containing galactose-alpha-1,3-galactose (α-gal) antigen can recruit and activate blood-derived macrophages to the injection site via the complement pathway. Recruited macrophages produced VEGF and dermal wounds demonstrated faster, scar-less healing. In this work, we use human microglia HMC3 cells as a model to determine whether a-gal based nanoparticles can similarly transform quiescent microglia towards a pro-healing phenotype. Early results indicates that α-gal nanoparticles (when activated with serum) increase CD68 expression in resting HMC3 cells. Morphological analysis of these cells show greater elongation and larger total cell area vs controls. a-gal treated HMC3 cells further increased CD206 and arginase expression. Functionally, the α-gal exposed cells produced less pro-inflammatory cytokines (TNF-α and IL-6) compared to cells incubated with IFN-γ. The cumulative results suggest that like their blood derived macrophage counterparts, CNS microglia exposed to activated α-gal nanoparticles may induce activation to a pro-healing state. These implications may justify using a-gal nanoparticles as a therapeutic for SCI.

Disclosures: B. Gopalakrishnan: None. **U. Galili:** None. **A. Dunbar:** None. **L. Solorio:** None. **R. Shi:** None. **J. Li:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.01

Topic: D.03. Somatosensation – Pain

Support: National Research Foundation of Ukraine Grant 2020.01/0266

Title: Vagus nerve signaling alteration during chronic airway inflammation.

Authors: *Y. TKACHENKO[1], A. SAVOTCHENKO[1], A. DZHUMA[1], M. FEDORIUK[1], M. STEFANENKO[1], S. DEMCHENKO[1], T. DREVYTSKA[2], O. USTINOV[1], R. MORHACHOV[2], D. ISAEV[1];
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Abstract: Patients with severe COVID-19 illness may become critically ill with the development of acute respiratory distress syndrome (ARDS). The aim of this study was to investigate the involvement of the nervous system in the regulation of immune responses, and how chronic airway inflammation may be altered by the application of the $TRPV_1$ receptor agonist capsaicin. We used the ARDS model induced by lungs hyperventilation with poly-I:C 15 mg/kg in 6 month-old male Wistar rats. After anesthesia and subdiaphragmatic vagotomy single-unit activity was recorded in the cervical vagus nerve of mechanically ventilated animals. The first three groups were mechanically ventilated with a high tidal volume of 35 ml/kg –Vt35 (hyperventilation) for 4 hours. Group I: rats were administered poly I:C by intratracheal injection before hyperventilation; capsaicin 20 μg/kg was injected in a venous catheter near the right atrium after two hours of hyperventilation. Group II: hyperventilation with poly I:C. Group III: only hyperventilation. Group IV: control group was mechanically ventilated with a fixed tidal volume of 10 ml/kg and for 4 hours. All rats were sacrificed after the experiment and lungs were preserved for immune and histology study. Primary afferents were classified into “regular” and “irregular” according to the pattern of their activity. The control group with constant normoventilation showed a constant frequency of spike discharges (3.48 ± 0.91) during the experiment. The activities of nerve fibers for Group I, II and III at control conditions before hyperventilation were 1.7 ± 0.6 (n = 20), 0.41 ± 0.17 (n = 16) and 8.34 ± 2.15 (n = 20) imp/sec respectively. Hyperventilation resulted in significant growth spike discharges frequency in all primary afferents for Group I, II, and III compared to control: to 6.68 ± 1.73, 3.35 ± 0.87 and 19.42 ± 3.99 imp/sec ($p < 0.05$). And not recovered to control values after a stop of hyperventilation and were for Group I, II and III: 6.12 ± 1.49, 2.42 ± 0.71 and 20.47 ± 5.12 imp/sec respectively ($p < 0.05$). Capsaicin (2 μM/kg) evoked action potential discharge of 100% (11/11) of tested vagal afferents with irregular activity, and only one (1/9) afferent with regular activity was activated by capsaicin 20 μg/kg during hyperventilation. Our data have shown that long-time lungs hyperventilation is the main excitatory factor for primary afferents. Capsaicin (20 μM/kg) evoked action potential discharge of 55% (11/20) of vagal afferents but does not have a noticeable effect on the excitability of nerve fibers during hyperventilation.

Disclosures: Y. Tkachenko: None. **A. Savotchenko:** None. **A. Dzhuma:** None. **M. Fedoriuk:** None. **M. Stefanenko:** None. **S. Demchenko:** None. **T. Drevytska:** None. **O. Ustinov:** None. **R. Morhachov:** None. **D. Isaev:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS107569

Title: Activation of spinal GPR109A attenuates spinal neuroinflammation and chronic pain in a lupus mouse model

Authors: *H.-R. WENG[1], V. VIATCHENKO-KARPINSKI[2], L. KONG[3];
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Abstract: Many patients with systemic lupus erythematosus (SLE) live with chronic pain despite advances in medical management in reducing mortality related to SLE. Few animal studies have addressed mechanisms and treatment for chronic pain caused by SLE. In this study, we provide the first evidence for the analgesic effects of a GPR109A specific agonist (MK1903) and its action mechanisms in chronic pain in female MRL/lpr mice, an SLE mouse model. We show that MRL/lpr mice at age 11 to 16 weeks had a higher sensitivity to heat stimuli. This was accompanied with significant microglial activation, increases in p38 MAPK and glutamatergic synaptic activities in the spinal dorsal horn. We demonstrate that chronic pain in MRL/lpr mice was significantly attenuated by intrathecal injection of MK1903. Activation of GPR109A receptors attenuated glutamatergic synaptic activity via suppressing production of interleukin-18 (IL-18). We provide evidence that activation of GPR109A attenuated chronic pain in the SLE animal model via suppressing p38 MAPK activity and production of IL-18. Our study suggests that targeting GPR109A is a potent approach for reversing spinal neuroinflammation, abnormal excitatory synaptic activity, and management of chronic pain caused by SLE.

Disclosures: H. Weng: None. **V. Viatchenko-Karpinski:** None. **L. Kong:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.03

Topic: D.03. Somatosensation – Pain

Title: Behavioural comparison of the UVB model of sterile inflammatory pain between Wistar rats and C57BL/J mice

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Transpharmation Ltd, Sandwich, United Kingdom

Abstract: Acute over-exposure of skin to ultraviolet (UV)-B radiation (as frequently occurs following sunburn) has emerged as a highly translatable pain model inducing both thermal and mechanical hyperalgesia in rats and humans (Bishop et al., 2007, 2009, 2010). This model is characterised by transient sensory alterations with peak effect between 24-48h post irradiation (Davies et al., 2011) that start to resolve 72h post exposure. The rat model developed by Bishop and colleagues closely mimics the hypersensitivity observed in the clinical setting and allows investigation of altered sensory and neurogenic effects in vivo. The aim of this study was to compare the effects of increasing intensities of UVB irradiation in thermal and mechanical hyperalgesia in Wistar rats and C57BL/J mice. Additionally, we tested the effect of acute oral treatment in thermal hyperalgesia and mechanical allodynia.
Studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. Under isofluorane anesthesia, the plantar area of adult male Wistar rats was exposed to 500, 750 and 1000mJ/cm^2 with UVB light (n=27); adult male C57BL/J mice were irradiated with 500, 1000 and 1250mJ/cm^2 (n=26). Mechanical allodynia and thermal hyperalgesia were measured using von Frey hairs and a hot plate, respectively. Hypersensitivity was measured before and on days 1, 2, 4 and 7 after UVB exposure. The effect of orally dosed naproxen (20mg/kg; n_{rats}=9) and indomethacin (10mg/kg; n_{mice}=9) as well as of the TRPV1 antagonist, SB366791 (0.5mg/kg; n_{rats}=9; n_{mice}=8), were assessed on day 2.
Overall, all the tested intensities were effective in reducing the mechanical and thermal thresholds of rats (mechanical $ANOVA_{RM}$, time: $F(20,398)=2.680$, $p<0.0001$; intensity: $F(5,398)=19.68$, $p<0.0001$; thermal $ANOVA_{RM}$, time: $F(4,206)=42.87$, $p<0.0001$; intensity: $F(2,206)=0.7771$, $p=0.4611$) and mice (mechanical $ANOVA_{RM}$, time: $F(4,243)=53.40$, $p<0.0001$; intensity: $F(2,243)=1.193$, $p=0.3050$; thermal $ANOVA_{RM}$, time: $F(4,282)=59.16$, $p<0.0001$; intensity: $F(2,282)=1.213$, $p=0.2990$), an effect that peaked 48h after the exposure to UVB and lasted until at least 4 days after UVB exposure. Post-hoc tests show that both pharmacological approaches significantly reversed mechanical allodynia in both rats and mice. However, only the TRPV1 antagonist, but not naproxen or indomethacin, was significantly effective in treating thermal hyperalgesia 48h after induction. These results point to a lack of efficacy of standard gold anti-inflammatory drugs in an important part of the profile of this particular model of sterile inflammatory pain and the need to develop targeted treatments for different pathologies.

Disclosures: A. David-Pereira: None. **C. Millum:** None. **C. Taylor:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.04

Topic: D.03. Somatosensation – Pain

Support: NIH NCATS TL1TR002531

Title: Longitudinal in vivo imaging and characterization of neuroinflammation after repetitive mild traumatic brain injury (mTBI) and its role in long-term pain.

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Abstract: Repetitive mild traumatic brain injury (mTBI) constitutes a major global health problem with neuropathological/behavioral sequelae contributing to long-term post traumatic pain (pTP). The prolonged neuroinflammation observed with mTBI is still poorly understood. To better understand the sequela of neuroinflammation following mTBI, we are assessing the degree to which inflammasomes contribute to the pathological response. The downstream events of this activating complex is known to initiate auto-activation of caspase-1 and consequent maturation of caspase-1 target molecules such as interleukin (IL)-1β and IL-18. In this study, we assessed the longitudinal changes of inflammasome activation in real-time by performing in vivo bioluminescence imaging (IVIS) of caspase1-activated-luciferase reporter mice. These mice were subjected to skull thinning and three repetitive controlled cortical impact (CCI) injuries separated by one week. First, we found an overall significant increase in caspase-1 activation after each mTBI, and this inflammatory enhancement was sustained for at least one month after three injuries. Second, we found greater lipopolysaccharide (LPS) – induced caspase-1 activation in injured mice cortical slices ex vivo compared to sham control. Furthermore, we also observed significant increases in astrocytic and microglial activation in these mice up to at least one week after the third mTBI. Pain behavioral assessments shows robust decrease in pain threshold (i.e. increased pain sensitivity) through Von Frey testing after three mTBIs, and this enhancement in pain sensitivity was sustained for almost two months post injury. There are also significant differences in inflammasome activation based on gender following the injury paradigm. Males were observed to exhibit rapid onset of caspase-1 activation following the first injury and a return to baseline one month after third mTBI. Female mice, exhibit reduced levels of caspase-1 activation following the first and second trauma events. However, caspase-1 activation levels were greatly enhanced after third mTBI and remained elevated for at least one month. Taken together, caspase-1 luciferase reporter mice, provide a unique tool to monitor neural inflammatory changes over time following cranial trauma. Our preliminary observations provide insights into the gender-specific responses which accompany repetitive mTBI-induced neuroinflammation, and its associated pain behaviors. Overall, this study provides a significant step in advancing our understanding of the short-term and long-term trauma-associated inflammatory events, which may contribute to mTBI-associated pTP.

Disclosures: T. Nguyen: None. **A. Cochran:** None. **S. Talley:** None. **E. Campbell:** None. **F.A. White:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.05

Topic: D.03. Somatosensation – Pain

Support: MRC UK DiMeN DTP Studentship (MR/N013840/1)

Title: The role of resolvins in chronic pain

Authors: *N. WONG[1,2], D. W. LAMBERT[1,2], F. M. BOISSONADE[1,2];
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Abstract: A series of findings suggests that resolvins, a class of molecules derived from omega-3 fatty acids, are instrumental in the active process of terminating inflammation. Recent studies have also shown that resolvins can alleviate pain symptoms in pre-clinical models, demonstrating their therapeutic potential in chronic pain. Since resolvins are naturally occurring and are produced endogenously, they may cause fewer adverse side effects than other therapeutics. Thus, resolvins have potential as pain therapeutics, and may help to address the unmet clinical need for effective and safe pain medications. Therefore, this study aimed to explore the role of resolvins in chronic pain, and how they interact to reduce inflammation and pain.

Dental caries can lead to inflammation in the tooth pulp and spontaneous pain, but not all patients with carious teeth report symptoms of pain. By comparing resolvin receptor expression in human tooth pulp tissues from patients with and without pain via immunohistochemistry, we hope to identify resolvin receptors that may be relevant to pain.

The specificity of antibodies we used was first evaluated using human umbilical vein endothelial cells (HUVECs), which have been previously shown to express the resolvin receptors BLT1, GPR32, ChemR23, and ALX/FPR2. The presence of BLT1, GPR32, and ChemR23 transcripts in HUVECs was confirmed using qPCR, and clear, positive immunocytochemical labelling for these receptors was present in the cells. BLT1 protein expression was also identified in HUVECs by western blot. To further validate BLT1 antibody specificity, immunocytochemistry and western blot assays were repeated after knocking down BLT1 expression in HUVECs via siRNA transfection. A significant reduction in BLT1 expression was observed compared with cells transfected with negative control non-targeting siRNA and mock transfection control, showing that the antibody is specific to BLT1.

The correlation between resolvin receptor expression and inflammation and pain in human tooth pulp tissues was then explored. To date, we have identified expression of BLT1 and GPR32 in human tooth pulp tissues using immunohistochemistry, and examined BLT1 expression in detail. BLT1 was found in blood vessels and nerve fibres, and further studies were conducted to quantify BLT1 expression in different groups of tooth pulp tissues. BLT1 expression in tissues from the carious painful group (n=11) was found to be significantly higher ($p<0.05$) than that

from the carious non-painful group (n=11), indicating that BLT1 may have a role in inflammatory pain. Ongoing work is examining BLT1 labelling in blood vessels and nerve fibres in human tooth pulp tissues.

Disclosures: N. Wong: None. **D.W. Lambert:** None. **F.M. Boissonade:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.06

Topic: D.03. Somatosensation – Pain

Support: NIH Grant GM115384

Title: Ketamine normalizes high-gamma power in the anterior cingulate cortex in a rat chronic pain model

Authors: *Q. ZHANG, I. FRIESNER, E. MARTINEZ, H. ZHOU, J. GOULD, A. LI, G. SUN, Z. CHEN, J. WANG;
NYU Sch. of Med., New York, NY

Abstract: Chronic pain alters cortical and subcortical plasticity, causing enhanced sensory and affective responses to peripheral nociceptive inputs. Previous studies have shown that ketamine had the potential to inhibit abnormally amplified affective responses of single neurons by suppressing hyperactivity in the anterior cingulate cortex (ACC). However, the mechanism of this enduring effect has yet to be understood at the network level. In this study, we recorded local field potentials from the ACC of freely moving rats. Animals were injected with complete Freund's adjuvant (CFA) to induce persistent inflammatory pain. Mechanical stimulations were administered to the hind paw before and after CFA administration. We found a significant increase in the high-gamma band (60-100 Hz) power in response to evoked pain after CFA treatment. Ketamine, however, reduced the high-gamma band power in response to evokedpain in CFA-treated rats. In addition, ketamine had a sustained effect on the high-gamma band power lasting up to five days after a single dose administration. These results demonstrate that ketamine has the potential to alter maladaptive neural responses in the ACC induced by chronic pain.

Disclosures: Q. Zhang: None. **I. Friesner:** None. **E. Martinez:** None. **H. Zhou:** None. **J. Gould:** None. **A. Li:** None. **G. Sun:** None. **Z. Chen:** None. **J. Wang:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 MH105528

Title: Changes in the dendritic spine density of the cortical neurons triggered by inflammatory pain

Authors: *E. DIMITROV, A. PAPADOGIANIS;
RFUMS, North Chicago, IL

Abstract: Background: The medial prefrontal cortex (mPFC) plays a major role in cognitive processes. Pain-associated impairment of cognitive functions is well established phenomenon but interestingly, female rodents are less affected. One possible explanation for pain-associated cognitive impairment is that pain alters the neuronal plasticity in the mPFC, as expressed by changes in the synaptic density of cortical neurons. Here, we compared the effect of inflammatory pain on the dendritic spine density of cortical neurons in male and female mice. Methods: Injection of Complete Freund's Adjuvant (CFA) into the hind paw served as a model for inflammatory pain. The neuronal soma, dendrites and spines were developed following Golgi-Cox impregnation. Microscopic Z-stack images at 100X magnification were collected bilaterally from the mPFC and were analyzed by ImageJ software. First, the neurons were categorized as pyramidal or interneurons according to their distinguish shape, size and dendritic arbor. The dendritic spines were counted on sections from third and fourth order dendrites. The spines were placed into 5 groups: mushroom, stubby, thin, long or branched. The counts of one to five dendrites per neuron on three to five neurons per animal were averaged by two independent observers in blind manner. Results: The CFA injection into the hind paw decreased the thermal threshold in both male and female mice. However, the CFA injection increased the dendritic spine density of the pyramidal neurons only in male mice. The increase of spine density was equal across different spine types. The CFA injection also increased the dendritic spine density of the interneurons in the mPFC of male mice, again without significant difference between the density of specific spine types. The CFA injection did not change the dendritic spine density of the pyramidal neurons in the mPFC of female mice but the injection did increase the spine density of the interneuronal dendrites. Again, as in males, the increase was equally distributed among the different spine types. Conclusion: Very likely, the resilience of female rodents to some of the deleterious cognitive effects of inflammatory pain relies on the unaltered spine density of the mPFC pyramidal neurons.

Disclosures: E. Dimitrov: None. **A. Papadogianis:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.08

Topic: D.03. Somatosensation – Pain

Support: F31AA028445
R01AA025996
T35AA021097

Title: Sex-dependent effects of alcohol on inflammatory pain and limbic system neuroadaptations in the context of pain

Authors: *J. A. CUCINELLO-RAGLAND, L. C. GOLD, S. EDWARDS, K. N. EDWARDS; Dept. of Physiol., Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Although a high percentage of both chronic pain patients and high-risk alcohol drinkers consume alcohol to manage their pain, there is a significant gap in knowledge regarding the mechanisms underlying the anti-nociceptive effects of alcohol. Previous findings from our lab suggest that the anti-nociceptive efficacy of alcohol may change over time in the context of chronic pain. The goals of the current project were to determine the longitudinal effects of alcohol on chronic pain and identify neuroadaptations associated with alcohol-induced anti-nociception. We utilized the complete Freund's adjuvant (CFA) model of inflammatory pain in adult female and male Wistar rats. Both nociceptive and negative motivational aspects of pain were measured using electronic von Frey (mechanical nociception), thermal probe test (thermal nociception), and mechanical conflict avoidance task (pain avoidance-like behavior). All behavioral tests were conducted at baseline and 1 and 3 weeks following intra-plantar CFA or saline administration. At both time points post-CFA, animals were treated with each of 3 doses of alcohol (intraperitoneal; 0g/kg, 0.5g/kg, 1g/kg) over separate days in a Latin square design. Alcohol produced dose-dependent analgesia in females but only modest anti-hyperalgesia in males. Consistent with clinical findings, only males displayed increased pain avoidance-like behavior, and this was most effectively attenuated by alcohol 1 week post-CFA. Although alcohol continued to attenuate CFA-induced decreases in both thermal and mechanical nociceptive thresholds in males 1 and 3 weeks post-CFA, females appeared to develop tolerance to these effects 3 weeks post-CFA. A separate cohort of rats was generated using these parameters to determine alterations in endocannabinoid (eCB) and glucocorticoid system-related protein levels in limbic regions, including the basolateral amygdala (BLA), central amygdala (CeA), and cingulate cortex. Western blot analysis revealed that alcohol differentially affects BLA and cingulate levels of the eCB synthetic enzyme diacylglycerol lipase-α (DAGLα) only in females. Preliminary data also suggests that alcohol decreases CeA glucocorticoid receptor (GR) phosphorylation only in control females, but not their CFA-treated counterparts. These findings will help elucidate the mechanism of analgesic action of alcohol in the context of chronic inflammatory pain states across sexes.

Disclosures: J.A. Cucinello-Ragland: None. **L.C. Gold:** None. **S. Edwards:** None. **K.N. Edwards:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.09

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01AR069951
NIH Grant R01DK122798

Title: The impact of neural activity on immune cell profile in the mouse colon

Authors: *A. EPOUHE[1], B. M. DAVIS[2], K. M. ALBERS[3];
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Abstract: The impact of neural activity on immune cell profile in the mouse colon
A.Y. Epouhe, B.M. Davis and K.M. Albers
Pain detecting sensory neurons are now recognized as contributors to the body's immune response under normal physiological and pathological conditions. In the colon, dysregulation of this communication is thought to contribute to the chronic and recurring inflammatory conditions of the gut evident in inflammatory bowel disorders (Crohn's and colitis). In this exploratory study we are using optogenetic strategies to probe how nerve activity impacts immune cell populations in the distal colon. Recent studies using pharmacological agonism and chemical ablation strategies have demonstrated the capacity of visceral nociceptors to sense bacteria and modulate the inflammatory response. These approaches provide new but limited insight into whether sensory afferents have a direct or indirect role in these processes. To address this issue, we are using mice that express the light-activated channelrhodopsin (ChR2) protein under control of the TRPV1 or MrgD promoter. Targeted optogenetic activation of sensory afferents that innervate the distal colon is done *in situ* using a fiberoptic probe inserted into the distal colon. Colon tissue is then isolated from stimulated mice and analyzed using immunolabeling of sectioned swiss roll and whole mount colon tissue to determine the relative number and location of MHCII+ antigen-presenting cells and CD3+ T cells in the colon tissue. Analysis of changes in muscle thickness and opposition of fibers to immune cells are also taken to assess signs of inflammation and association between neurons and immune cells. Comparison of two groups of mice is being done: Group 1 mice express ChR2 in TRPV1+ or MrgD+ neurons and receive optogenetic stimulation and Group 2 mice that express ChR2 in TRPV1+ or MrgD+ neurons but do not receive optogenetic stimulation. Mice utilized for these experiments were on a C57/Bl6 background and were between 8-24 weeks old. Both male and female mice were included for

analysis, but sex differences were not assessed. Mice were stimulated for two 30 min sessions, with 2 hour breaks, over with a final 2 hour break prior to tissue collection. Initial results indicate that light stimulation of TRPV1+ colon afferents decreases the number of MHCII+ cells in the lamina propria of the colon, based on level of analysis with little or no change in CD3+ cell number. This suggests that acute TRPV1 stimulation may lead to MHCII+ cell migration in the distal colon. These studies were supported by NIH grant 5R01AR069951 and R01DK122798.

Disclosures: A. Epouhe: None. **B.M. Davis:** None. **K.M. Albers:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.10

Topic: D.03. Somatosensation – Pain

Title: Quantitative sensory testing in hidradenitis suppurativa could provide insight into pain mechanisms

Authors: A. M. ALSOUHIBANI[1,3], J. R. BARRON[2], M. GUNTER[1], O. G. HOEKENDIJK[1], P. SPECK[2], E. F. COLE[2], L. A. V. ORENSTEIN[2], *D. E. HARPER[1];
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Abstract: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease that is characterized by recurrent nodules, abscesses, and scarring tunnels. Most people suffering from HS report moderate or worse pain, contributing to reduced quality of life. The aim of our study was to determine the primary mechanism underlying the pain in individuals with HS using quantitative sensory testing (QST).
QST was performed in individuals with HS using the standardized protocol by the German Research Network on Neuropathic Pain (DFNS). The skin locations tested by QST included: (1) the dorsal hand (control site), (2) an actively inflamed HS lesion, and, if present, (3)a non-inflamed HS lesion. The tests included were cold, warm, and mechanical detection thresholds (CDT, WDT and MDT); cold, heat, and mechanical pain thresholds (CPT, HPT and MPT); thermal sensory limen (TSL); paradoxical heat sensations (PHS); pressure pain thresholds (PPTs); dynamic mechanical allodynia (DMA); mechanical pain sensitivity (MPS); and windup ratio (WUR).
Seven individuals with HS (age=39.7±12.5 years; 5 women) diagnosed by a dermatologist participated in the study. A total of 10 skin locations were tested by QST - 5 draining tunnels (inflamed), 2 scarred nodules (non-inflamed), 1 inflammatory nodule, 1 non-inflamed tunnel, and 1 perilesional site(s). The frequency of participants who had abnormal QST values (defined as a value greater than 95% confidence interval of the reference data) at the control site are as

follows: Hyperalgesia (gain of function): WUR = 2/7 (29%), MPS = 4/7 (57%), DMA = 3/7 (43%), PPTs = 3/7 (43%); Hypoesthesia (loss of function): WDT = 1/7 (14%), CDT = 1/7 (14%). All other measures (i.e., MPT, TSL, CPT, HPT, PHS) were within normal range. One participant (14%) reported paradoxical sensations at an inflamed location (draining tunnel). Also, a high frequency of abnormalities in the thermal sensory limen (TSL), indicative of small fiber loss, were found: 5/6 (83%) at the inflamed location and 3/4 (75%) at the non-inflamed location. Preliminary results suggest that peripherally, at the affected skin, a neuropathic component could play a major role in the perception of pain. However, the pain experienced by a subset of individuals may involve more than the pathology at the skin lesion and could be characterized as nociplastic. Other aspects such as descending inhibitory pathways should be investigated in future studies.

Disclosures: A.M. Alsouhibani: None. **J.R. Barron:** None. **M. Gunter:** None. **O.G. Hoekendijk:** None. **P. Speck:** None. **E.F. Cole:** None. **L.A.V. Orenstein:** None. **D.E. Harper:** None.

Poster

P396. Pain Models: Inflammatory Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P396.11

Topic: D.03. Somatosensation – Pain

Support: University of Pennsylvania startup funds
Columbia University startup funds
NIH R00-DE026807
The Rita Allen Foundation
The Alfred P. Sloan Foundation

Title: Unlocking the behavioral signatures of inflammatory pain at millisecond resolution with supervised and unsupervised feature extraction

Authors: A. JHUMKA[1], ***W. FOSTER**[1], N. MOSSAZGHI[2], E. A. YTTRI[2], I. ABDUS-SABOOR[1];
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Abstract: Chronic pain accounts for up to 20% of physician visits and as many as two thirds of patients are unsatisfied with current treatments. While humans can describe their pain levels both qualitatively and quantitatively, studies on pain in rodents have long relied on assays providing incomplete behavioral information. Discovering effective analgesics therefore depends on our ability to provide more objective and comprehensive measurements of pain in laboratory animals. To that end, we combine automated paw tracking with two statistical software platforms

to extract pain-related behavioral features in mice. PAWS (Pain Assessment at Withdrawal Speeds), uses a univariate projection of hind paw center position over time to automatically quantify predefined behavioral features of the hind paw withdrawal response. BSOiD (Behavioral Segmentation of Open-field In DeepLabCut) is an unsupervised learning algorithm that reveals and classifies behavioral features which are not user-defined by performing novel expectation maximization fitting of Gaussian mixture models on t-Distributed Stochastic Neighbor Embedding (t-SNE). In combination, these approaches generate a complete and intuitive description of hind paw withdrawal behavior on a trial-by-trial basis. While we previously described the behavioral signatures of acute evoked pain with PAWS, the signatures of inflammatory pain remained elusive. Here, we use PAWS and BSOiD to determine the behavioral signatures of carrageenan induced inflammatory pain and subsequent treatment with a nonsteroidal anti-inflammatory drug (meloxicam) in n=10-20 male C57BL/6J mice. With PAWS, we find that carrageen elicits changes in behavior indicative of increased mechanical sensitivity and that subsequent treatment with meloxicam tempers this increase in a behavior-feature specific fashion. With BSOiD, we make a novel distinction between angled and flat hind paw guarding behavior, which are selectively upregulated during mechanical allodynia and mechanical hyperalgesia respectively. To date, this measurement is the only specific behavioral readout that distinguishes between mechanical allodynia and hyperalgesia. Thus, our methods provide a framework to rigorously assess pain-related behaviors, and pain sensation by proxy, in multitudes of experimental paradigms.

Disclosures: A. Jhumka: None. **W. Foster:** None. **N. Mossazghi:** None. **E.A. Yttri:** None. **I. Abdus-Saboor:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.01

Topic: D.09. Multisensory Integration

Support: Swedish Research Council
Göran Gustafssons Foundation
Hjärnfonden
Wenner-Gren Foundation

Title: Inference of a common cause for body ownership takes trial-to-trial sensory uncertainty into account

Authors: *M. CHANCEL[1], H. H. EHRSSON[1], W. MA[2];
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Abstract: How do we perceive the fundamental distinction between the physical self and the external world, and how do we come to sense that an object in view, for example, a hand, is part of our own body or not? Over the past two decades, many studies have investigated the contributions of vision, touch, and proprioception to the sense of body ownership, i.e., the multisensory perception of limbs and body parts as our own. However, the processes involved in subjectively experienced body ownership have only been qualitatively described, and the computational principles that determine such sensations remain unclear. To address this issue, we developed a detection-like psychophysics task based on the classic rubber hand illusion paradigm where participants were asked to report whether the rubber hand felt like their own (the illusion) or not. We manipulated illusion strength by varying the asynchrony of visual and tactile stimuli delivered to the rubber hand and the hidden real hand under different levels of visual noise. We found that (1) the probability of the emergence of the rubber hand illusion increased with the addition of visual noise and was well predicted by a causal inference model involving the observer computing the probability of the visual and tactile signals coming from a common source; (2) the causal inference model outperformed a non-Bayesian model involving the observer not taking into account sensory uncertainty; and (3) by comparing the casual inference of ownership and visuotactile synchrony detection, we found that the prior probability of inferring a common cause for the two types of multisensory precepts was correlated but greater for ownership, which suggests that individual differences in rubber hand illusion can be explained at the computational level as differences in how priors are used in the multisensory integration process. Collectively, these results demonstrate that the sense of body ownership is determined by Bayesian causal inference, which implies that the same statistical principles determine the perception of the bodily self and the external world.

Disclosures: M. Chancel: None. **H.H. Ehrsson:** None. **W. Ma:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.02

Topic: D.09. Multisensory Integration

Support: Swedish Research Council Grant 2017-0315

Title: Body ownership and the neural processes of memory encoding and reinstatement

Authors: *H. M. IRIYE[1], H. H. EHRSSON[2];
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Abstract: Memories are inextricably tied to having a body. Yet, we are just beginning to understand how the fundamental perception of one's body as one's own (i.e. body ownership)

influences neural processes during encoding and retrieval. Here, we investigated how body ownership influences patterns of neural activity associated with memory formation and reactivation during retrieval. We immersed participants (N = 27) within pre-recorded videos viewed through virtual reality glasses, which depicted realistic, everyday events that included a natural first-person view of a mannequin's reclining body which was aligned with the location of the participants' real bodies during fMRI scanning. We manipulated feelings of body ownership over the mannequin using a perceptual full-body illusion. Participants saw an object touch the mannequin and simultaneously felt touches on the corresponding location of their real body, which created an illusory sense of ownership over the mannequin. As a control condition, we disrupted the illusion by delivering seen and felt touches in an alternating pattern in half of the videos. The experimenter was blind to the condition assigned to each video. 1 week later, participants retrieved memories for the videos during fMRI scanning. Participants answered cued recall questions concerning specific event details at the end of each session to assess memory accuracy. Preliminary results suggest that patterns of activity during encoding in lateral frontal, medial and lateral temporal, posterior parietal, and visual cortices were able to differentiate between memories encoded with differing levels of body ownership. Further, by correlating patterns of activity at encoding and retrieval, we found that forming memories with intact compared to disrupted body ownership increased reinstatement of that event during retrieval in the hippocampus based on both the strength of perceived ownership over the mannequin during encoding and the accuracy with which event details were retrieved. Our findings have the potential to provide new insights into how to optimize memory for past events and preserve the ability to vividly relive the past in the context of both healthy ageing and clinical disorders (e.g., Alzheimers Disease).

Disclosures: H.M. Iriye: A. Employment/Salary (full or part-time):; Karolinska Institutet. **H.H. Ehrsson:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.03

Topic: D.09. Multisensory Integration

Support: Vetenskapsrådet (Swedish Research Council)
Göran Gustafssons stiftelse

Title: The neural substrates of body ownership and agency and their interaction during voluntary action

Authors: *Z. ABDULKARIM[1], A. GUTERSTAM SHAHIM[1], Z. HAYATOU[2], H. EHRSSON[1];
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Abstract: Background: The sense of body ownership and the sense of agency are two central concepts of bodily self-consciousness. Multiple neuroimaging studies have investigated the neural correlates of the senses of body ownership and agency in isolation, while few have investigated their relationship during voluntary movement. In this study we investigated the neural correlates of body ownership and the sense of agency as well as their interaction.
Methods: By eliciting the moving rubber hand illusion in 29 healthy volunteers while manipulating the synchrony of the finger movements of the rubber hand and the real hand, the movement type (active or passive movements) and the placement of the rubber hand (anatomically congruent or incongruent) during functional magnetic resonance imaging, we isolated activations reflecting the sense of body ownership and agency, respectively, as well as their interaction. This 2x2x2 factorial design allowed us to effectively control for confounds while designing contrasts that isolate the phenomena of interest. The statistical analysis was done using SPM12, and the main results are reported after family-wise error (FWE) correction with the alfa-level set at 0.05.
Results: We found that perceived ownership of the hand was associated with activity in premotor, posterior parietal and cerebellar regions whereas the sense of agency over the hand was related to activity in the superior temporal cortex and dorsal premotor cortex. Importantly, agency over a hand that felt like part of one's own body was associated with stronger activation in the somatosensory cortex than agency over a hand that felt like an external object, whereas activation in the visual association cortex was observed for the inverse relationship. We further noted that activations in previously identified agency regions such as the left insular cortex and right temporoparietal junction (angular gyrus) reflect the synchrony or asynchrony of the visuo-proprioceptive stimuli rather than the sense of agency.
Conclusions: These results identify the neural bases for the sense of agency and the sense of body ownership during voluntary movement, as well their interaction, and further suggest that the sense of agency over one's own bodily movements should be distinguished from agency over external events that are indirectly caused by one's actions.

Disclosures: Z. Abdulkarim: None. **A. Guterstam Shahim:** None. **Z. Hayatou:** None. **H. Ehrsson:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.04

Topic: D.09. Multisensory Integration

Title: Body ownership manipulation through a Multisensory platform combining virtual reality and nerve stimulation

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Abstract: We experience the environment through multiple senses, which often provide us with redundant information. Our brain uses this redundancy to merge the incoming information in an unambiguous perception. This process, known as multisensory integration, is fundamental to develop a comprehensive representation of what we consider our *self*. Even if these mechanisms are automatic, several neurological diseases cause changes in this coherent representation and lead patients to distorted perceptions of their body. Fortunately, this representation is malleable and can be manipulated. Evidence for this comes from the Full Body Illusion (FBI), where subjects develop ownership over a mannequin thanks to spatio-temporal congruent stimulation of the real and fake body. Numerous studies have replicated this illusion. However, the vast heterogeneity in the measurement approaches make it difficult to compare the results and shed light on the validity of the metrics. Moreover, the functional benefits of such approaches for rehabilitation purposes are not yet clear. In this project, we developed a multisensory platform combining two technologies that allow an unseen precision in the generation of the illusion: visual feedback through Virtual Reality (VR) and touch through Transcutaneous Electrical Nerve Stimulation (TENS). The subject is immersed in a realistic environment, where visual and tactile stimuli are synchronized in a millisecond precision. Unlike previous approaches, not only the platform allows to obtain high temporal precision, but also to stimulate multiple body parts and to perform any movement concomitantly. Importantly, we applied a multifaceted evaluation approach combining variate, objective and subjective measurements (e.g., proprioceptive drift, peri-personal space, questionnaires) to calculate the strength of the illusion. To validate our multimodal approach, we perform experiments recreating the FBI and using the same platform in a different scenario to understand the functional benefits of the visuo-tactile stimulation. The system is being tested in healthy subjects and patients affected by diabetic neuropathy. Our preliminary results show that the platform outdoes classical approaches and give indications on the validity of the measurements, confirming the inability of subjective reports to measure the illusion. Our platform stands as a new approach to perform body illusions with a highly reproducible and controlled method. Finally, the ability to stimulate distal areas with TENS and to adapt to the subject movements will allow it to be a new actor in the rehabilitation field to provide sensory, motor and cognitive benefits.

Disclosures: G. Preatoni: None. **F. Dell'Eva:** None. **A. Pedrocchi:** None. **S. Raspopovic:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.05

Topic: D.09. Multisensory Integration

Support: ERC Grant MYSpace 948349

Title: Task-specific cortical organization of multisensory spatial representation

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Abstract: For a long period, primary sensory cortices were thought to process unisensory information only. However, more recent literature highlighted a multisensory nature of primary sensory areas, suggesting that the design principle of the cortical architecture is task-specific rather than modality-specific. In agreement with this idea, early strong involvement of visual occipital areas has been shown for complex spatial representations, independently of the sensory modality involved. Given these assumptions, we hypothesized that in the processing of multisensory spatial information, visual occipital areas are recruited differently according to the nature of the spatial tasks. To explore this hypothesis, we tested healthy participants in an electrophysiological study involving two different spatial tasks. In a spatial bisection task, three audiovisual (AV) stimuli (S1, S2 and S3) were consecutively played at three different spatial positions and participants evaluated whether S2 was spatially farther from S1 or S3. In a spatial localization task, one AV stimulus (S2) was played in a specific spatial location and participants identified whether it was positioned on the left or the right of the setup's center. It is important to note that the AV stimulus to localize (S2) was identical between the spatial bisection and the spatial localization tasks (in terms of spatial position and sensory modalities), and that the only difference between the two was the experimental question. While participants performed the tasks, we measured their Event-Related Potentials (ERPs), focusing on responses in an early time window (50-90ms) known to be involved in the earliest stages of multisensory integration. As expected, in both tasks, S2 elicited an occipital and a temporal activation contralateral to its spatial position. Nevertheless, while the two tasks shared a similar activation to AV stimuli at temporal sites (taken as control areas), the occipital response during the spatial bisection task was significantly stronger than during the spatial localization task. As predicted, we revealed that the nature of the spatial task modulated the occipital activation to multisensory stimuli. Thus, the early recruitment of occipital areas might play a more important role in scaffolding the multisensory processing for constructing complex spatial representations, as in the spatial bisection task, while it might be less substantial in a simpler spatial localization task. Our study allows to further understanding the multisensory brain's supra-modal architecture and provides insights on how primary sensory areas encode task specificity.

Disclosures: G. Bertonati: None. **M. Amadeo:** None. **C. Campus:** None. **M. Gori:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.06

Topic: D.09. Multisensory Integration

Support: Dutch Province of Limburg
NWO Grant 451-15-012
NIH Grant RF1MH116978

Title: Cortical Depth-dependent Multisensory and Attentional Influences on Peripheral Sound Processing

Authors: *I. ZULFIQAR[1], E. FORMISANO[1], S. KASHYAP[2], P. DE WEERD[1], M. MOEREL[1];
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Abstract: Recent evidence supports the existence of multisensory processing in early auditory regions of the cortex[1,2,3]. To study the source of these multisensory modulations, we investigated visual influences on the auditory cortex in a cortical depth-dependent manner using high resolution functional MRI at 7 Tesla. Specifically, given the reciprocal connectivity between early visual cortex representing the periphery and auditory cortex[4,5], we set out to explore audiovisual integration of peripherally presented stimuli. For 10 subjects, we collected anatomical data at 0.6 mm isotropic and functional data at 0.8 mm isotropic resolution. In a blocked design, the participants were presented with unisensory stimuli and audiovisual stimuli [auditory (noise) and visual (grating, spanning 5 - 8^{o}); temporally modulating at 3 Hz; located at 5^{o} in the right azimuthal plane]. Attention was directed either towards the auditory stimulus, or away from both stimuli towards the fixation cross. Our preliminary results showed multisensory enhancement in a cortical network comprising early sensory sites (auditory and visual cortex), insula, and ventrolateral prefrontal cortex. Multisensory enhancement was present in the primary auditory cortex and increased along the auditory cortical hierarchy irrespective of attention. These findings confirm that the primary auditory cortex is not uniquely unisensory. Additionally, we observed a task-dependent attentional modulation of multisensory enhancement in the deep layers of the auditory belt. This suggests a top-down origin of attention that stems from long-range cortico-cortical feedback, which may originate from the visual cortex or higher-order association areas. Our finding contrasts the existing empirical observation of context-dependent feedback being most prominent in superficial layers due to a short-range cortical influence[3]. This difference in observations between studies indicates that the multisensory effects may critically depend upon the stimulus characteristics (location, salience, etc.), and tasks. Future analyses will include cortical depth-dependent connectivity analysis which may help discriminate between the frontal regions and visual cortex as sources of the observed context-dependent multisensory

enhancement in deep layers of the auditory belt, and a multivariate analysis to increase sensitivity of our analysis by examining distributed multisensory effects.
[1]Besle et al., 2008. J. Neurosci. [2]Kayser et al., 2008. Cereb. Cortex. [3]Gau et al., 2020. eLife. [4]Falchier et al., 2002. J. Neurosci. [5]Falchier et al., 2010. Cereb. Cortex.

Disclosures: I. Zulfiqar: None. **E. Formisano:** None. **S. Kashyap:** None. **P. de Weerd:** None. **M. Moerel:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.07

Topic: D.09. Multisensory Integration

Support: R35NS122333-01

Title: Integration of global and local features in stereognosis

Authors: *E. BERGER-WOLF, N. SHELCHKOVA, D. SHEETS, E. OKOROKOVA, A. R. SOBINOV, C. M. GREENSPON, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: When we grasp an object, we experience a vivid sense of its three-dimensional shape, a sensory ability termed stereognosis. At each point of contact with the object, tactile signals convey information about its local contours, for example, a curved surface or an edge. The global shape of the object is computed based on proprioceptive signals about the conformation of the hand. These two streams of information — cutaneous and proprioceptive — are then integrated to give rise to stereognosis, a process about which virtually nothing is known. In the present study, we sought to characterize how the local features and global shapes of objects interact at a perceptual level. To this end, human participants were asked to rate the dissimilarity of pairs of objects that differed in their local features, their global shape, or both. We found that the perceived dissimilarity of an object that varied in both local features and global shape could not be straightforwardly predicted from the dissimilarity of objects that varied only in one or the other. We explore the implications of our findings for the neural mechanisms of stereognosis.

Disclosures: E. Berger-Wolf: None. **N. Shelchkova:** None. **D. Sheets:** None. **E. Okorokova:** None. **A.R. Sobinov:** None. **C.M. Greenspon:** None. **S.J. Bensmaia:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.08

Topic: D.09. Multisensory Integration

Support: DFG SFB/TRR-135
DFG IRTG-1901
HMWK (TAM)

Title: Task-relevance reverses the effect of efference copy based predictions during multisensory path-integration

Authors: *L. ROSENBLUM[1], A. KREß[1], B. ARIKAN[2], B. STRAUBE[3], F. BREMMER[1]; [1]Dept. Neurophysics, Philipps-University Marburg, Marburg, Germany; [2]Dep. of Psychology, Justus Liebig Univ. Giessen, Giessen, Germany; [3]Translational Neuroimaging Marburg, Dept. of Psychiatry and Psychotherapy, Philipps-Universität Marburg, Marburg, Germany

Abstract: Successful navigation requires an accurate estimate of one's own movement through space. Information about self-motion parameters like travelled distance (path integration) is provided by various sensory modalities. Two important sources of information are retinal optic flow in the visual, and tactile flow across the skin in the tactile domain. To control our own movements, we must also distinguish self-generated from externally induced motion signals. The predictive coding framework suggests that the sensory representation resulting from one's own motion can be attenuated. In a previous fMRI study we tested this idea in a visual-auditory path integration task. We observed both supramodal BOLD suppression as well as modality-specific enhancement in sensory areas for active distance reproduction (Krala et al., JoV, 2019). In line with previous literature, we have proposed that for successful distance reproduction, task-relevance and prediction interacted synergistically, leading to enhanced instead of suppressed neural activation in early sensory cortices. To probe this hypothesis, task-relevance was manipulated in the current study. We explored the role of visual and tactile flow in the processing of self-motion information in a multisensory path integration task. In one block of trials, subjects' task was to replicate (Active/Reproduction = ACT/R) a previously observed distance of passive self-displacement (Passive/Reproduction = PAS/R). In another block, subjects first travelled a self-chosen distance (Active/Self = ACT/S) which was then played back to them (Passive/Self = PAS/S). Stimuli were presented in each unimodal and a bimodal condition: Visual self-motion was simulated as forward motion across a 2D ground plane of random dots. Tactile self-motion stimuli were delivered by air flow across the subjects' forehead. Cues from both modalities were combined congruently in the bimodal condition. In line with our previous study, preliminary results show higher BOLD activation of primary sensory cortices for visual (V1) and tactile (SI) self-motion cues for ACT/R as compared to PAS/R trials in all three modalities. As hypothesized, BOLD activation in these areas was suppressed for ACT/S as compared to ACT/R, which confirms that the effect of one's own action - and hence prediction - on sensory processing is strictly dependent on the respective behavioral task and its relevance.

Remarkably, for PAS/S compared to ACT/S we found stronger activation of primary sensory areas and higher-order sensory areas (e.g. insula) indicating that task-relevance can reverse the suppressing impact of prediction on sensory processing in a path integration task.

Disclosures: L. Rosenblum: None. **A. Kreß:** None. **B. Arikan:** None. **B. Straube:** None. **F. Bremmer:** None.

Poster

P505. Cross-Modal Processing In Humans II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P505.09

Topic: D.09. Multisensory Integration

Support: Region Bourgogne Franche Comté ANER RobotSelf

Title: Sensory-motor plasticity in the representation of self induced by robotic telepresence

Authors: *J. VENTRE-DOMINEY, D. FARIZON, P. F. DOMINEY;
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Abstract: The current research investigates the plasticity of the representation of self. The integrated perception of self is constructed through the multi-modal integration of sensorimotor signals including visual, proprioceptive, and tactile. The perception of self is plastic, and can be modified by substituting perceptual inputs. This has been classically revealed in the rubber hand illusion (Botvinick and Cohen 1998). A crucial aspect of this effect is the synchrony between visual and tactile stimulation. Interestingly this illusion can be extended to the whole body. We previously characterized a form of rubber hand extended to the whole body, using a form of motor synchrony (Ventre-Dominey, Gibert et al. 2019). In the current research we extend this and demonstrate the plasticity of the representation of self which can be evoked via tactile stimulation Using a simple neuroscience-inspired procedure to beam human subjects into robots, we previously demonstrated by visuo-motor manipulations that embodiment into a robot can enhance the acceptability and closeness felt towards the robot. In that study, the feelings of likeability and closeness toward the robot were significantly related to the sense of agency, independently of the sensations of enfacement and location. Here, using the same paradigm we investigated the effect of a purely sensory manipulation on the sense of robotic embodiment associated to social cognition. Wearing a head-mounted display, participants saw the visual scene captured from the robot eyes. By positioning a mirror in front of the robot, subjects saw themselves as a robot. Tactile stimulation was provided by stroking synchronously or not with a paintbrush the same location of the subject and robot faces. In contrast to the previous motor induction of embodiment which particularly affected agency, tactile induction yields more generalized effects on the perception of ownership, location and agency. Interestingly, the links

between positive social feelings towards the robot and the strength of the embodiment sensations were not observed. We conclude that the embodiment into a robot is not sufficient in itself to induce changes in social cognition and that the intentional motor signal constitutes an essential cue to link embodiment and social features.

Disclosures: J. Ventre-Dominey: None. **D. Farizon:** None. **P.F. Dominey:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.01

Topic: F.04. Stress and the Brain

Support: Hans Fischer Senior Fellowship-IAS-TUM. Munich
Klinikum rechts-der-Isar. TUM-Munich

Title: Epigenome-wide associations with maternal stress phenotypes in newborn saliva: FELICITy study.

Authors: R. SHARMA[1], C. ZELGERT[1], P. ZIMMERMANN[1], B. FABRE[2], R. WILSON[3], M. WALDENBERGER[3], J. W. MACDONALD[4], T. BAMMLER[4], M. G. FRASCH[5], S. M. LOBMAIER[1], *M. C. ANTONELLI[6];
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Abstract: Maternal stress before, during and after pregnancy have profound effects on the development and life-long function of the infant's stress response systems such as the Hypothalamic Pituitary Adrenal axis (HPA) and the Autonomic Nervous System (ANS). We hypothesized that prenatal stress (PS)-induced programming of HPA and ANS is reflected in epigenetic and biophysical biomarkers, which can be employed for early detection and follow up of affected children. In this presentation we will show results aimed to find non-invasive epigenetic biomarkers of PS in the newborn salivary DNA. Pregnant women were screened for stress exposure using Cohen Perceived Stress Scale (PSS-10) and were classified into stressed (SG, PSS-10 ≥ 19, n= 79) and control group (CG, PSS-10 <19, n=85) matched 1:1 for parity, maternal age and gestational age. Prenatal Distress Questionnaire (PDQ) was also administered to assess specific pregnancy worries. Maternal and fetal ECGs were recorded to derive coupling between maternal and fetal heart rates resulting in a Fetal Stress Index (FSI). Upon delivery,

maternal hair strands were collected for cortisol measurements and newborn's saliva samples were collected. DNA was extracted from saliva samples (n=114) and DNA methylation was measured using EPIC Bead-Chip array (850k CpG sites). To identify associations between PSS/PDQ/FSI/Cortisol and methylation, linear regression models adjusting for confounders (sex, gestational age, smoking, autoimmune disease, gestational diabetes, Illumina plate factor and surrogate variables (SVA)) were run using limma package in R. The top hits were consistent with the basic model adjusted for sex, gestational age, Illumina plate factor and SVAs. We found epigenome-wide significant associations for 6 CpG sites in association with stress phenotypes (PDQ and cortisol) at FDR < 5%. Annotated genes to the CpGs were found to be enriched for development and growth in hippocampus signalling pathway, organelle biosynthesis, and metabolism of proteins, cell proliferation and bone development. We report novel associations between DNA methylation and maternal stress phenotypes. We observe that the several associations are related to neurobiological disorders such as autism, schizophrenia, neural tube defects and atrophy. Since this is a longitudinal study, we are assessing the neurodevelopmental outcome of the two years old infants and we will assess the differential methylation in saliva samples at that age. Detecting differences in methylation between the two ages will give a window of opportunity for early interventions.

Disclosures: R. Sharma: None. **C. Zelgert:** None. **P. Zimmermann:** None. **B. Fabre:** None. **R. Wilson:** None. **M. Waldenberger:** None. **J.W. Macdonald:** None. **T. Bammler:** None. **M.G. Frasch:** None. **S.M. Lobmaier:** None. **M.C. Antonelli:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.02

Topic: F.04. Stress and the Brain

Support: 5R01MH117004-02

Title: Differential DNA methylation targets exons and CpG shores in the ventral dentate gyrus following early life adversity in mice

Authors: *E. BRINDLEY[1], A. NABILA[1], F. TAKI[1], S. ODELL[1], L. J. ZALLAR[1], M. SKELLY[1], J. GAL TOTH[1], R. J. CHEN[1], A. ALONSO[1], K. E. PLEIL[2], M. TOTH[3]; [2]Dept. of Pharmacol., [1]Weill Cornell Med., New York, NY; [3]Weill Cornell Med. Col., New York, NY

Abstract: Early life adversity (ELA) is known to cause psychopathological consequences later in life, such as anxiety in humans and anxiety-like behavior in rodent models. The adult behavioral manifestations of ELA suggest persistent epigenetic changes established during early

life in neurons implicated in anxiety-related behavior, including ventral dentate granule cells (vDGCs). Previous work identified small genomic regions containing clusters of differentially methylated CpG sites established during development. Here, we investigate how ELA during fetal development disrupts DNA methylation patterns in vDGCs by two ELA conditions resulting in innate anxiety-like behaviour in offspring: Maternal anxiety (MA), wildtype offspring of 5HT-1A deficient dams and Maternal LPS (ML), offspring of LPS injected dams. Using reduced-representation bisulfite sequencing, we observe differentially methylated CpG sites clustering to 5364 small genomic regions following ELA. These differentially methylated regions (ELA-DMRs) were defined as 2CpG sites with >5% methylation change within 1kb, present in MA and ML ELA conditions with at least 1 bp overlap. ELA-DMRs were enriched in exonic regions and CpG shores, and less prominently in islands. Further, exonic localization of ELA-DMRs indicates preferential targeting of evolutionarily conserved sequences by ELA. At the CpG level, there was a close to 50:50 split between hypomethylation and hypermethylation. Single cell RNA sequencing of vDGCs from MA mice, revealed 124 differentially expressed genes with respect to control. 63 of these 124 genes contained one or more ELA-DMRs, suggesting a correlation between DNA methylation and gene expression. Functions related to synaptic transmission were overrepresented in this subcategory and 94% were downregulated in ELA with respect to control. Many of the differentially expressed genes that did not contain DMRs were related to potassium channels and 80% were upregulated in ELA with respect to control. Decreased expression of synaptic genes and increased expression of potassium channels are consistent with preliminary electrophysiological experiments indicating a reduction in excitability of vDGCs.

Disclosures: E. Brindley: None. **A. Nabila:** None. **F. Taki:** None. **S. Odell:** None. **L.J. Zallar:** None. **M. Skelly:** None. **J. Gal Toth:** None. **R.J. Chen:** None. **A. Alonso:** None. **K.E. Pleil:** None. **M. Toth:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.03

Topic: F.04. Stress and the Brain

Support: NIH Grant 1R01HD087509-01

Title: Mitigating the epigenetic consequences of early life stress with a behavioral intervention

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Abstract: Aversive caregiving in early life is a risk factor for aberrant brain and behavioral development throughout the lifespan. This outcome is related to aberrant epigenetic regulation of the *Bdnf* gene. The *Bdnf* gene encodes for brain-derived neurotrophic factor (BDNF), a neurotrophin involved in neural plasticity, learning, and memory. Disruptions in caregiver-infant interactions lead to increased methylation and decreased expression of the *Bdnf* gene. One way to mitigate this effect is hypothesized to be through aerobic exercise. Exercise increases BDNF at the protein and gene expression levels, making it an exciting target for therapeutic interventions. To our knowledge, exercise has never been studied as a therapeutic intervention in preclinical rodent models of early life caregiver maltreatment. To that end, the current study is investigating the effect of an adult voluntary wheel running intervention on *Bdnf* aberrant methylation and expression in the prefrontal cortex of rats who experienced aversive caregiving in infancy. We are using a rodent model (Long Evans rats) wherein rat pups experience intermittent caregiver-induced stress from postnatal days 1-7 and are given voluntary access to a running wheel (except in the control condition) from postnatal days 70-90 as a young adulthood treatment intervention. This study presents novel findings on the mechanisms underlying neural plasticity in the prefrontal cortex in an early life stress context. Current results suggest that early life stress and exercise affect *Bdnf* gene methylation and expression in an exon and sex specific manner. Future work will continue to probe sex differences in wheel running behavior and epigenetic activity.

Disclosures: T. Campbell: None. **K. Donoghue:** None. **N. Collins:** None. **S. Fern:** None. **H. Duffy:** None. **T.L. Roth:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.04

Topic: F.04. Stress and the Brain

Support: NIH-NINDS Child Neurologist Career Development Program K12NS098482
UCI-ICTS Pilot Project Grant
Conte Center Seed Grant
Robert Wood Johnson Foundation Amos Award

Title: Neuronal transcriptomic and epigenomic signatures of early-life adversity and exercise underlie modulations to cognitive function

Authors: *N. E. NELSON[1], B. GOMRINGER[2], S. D. A. VALIENTES[3], P. ABDOLI[2], A. BAYAT[3], C. MANDIA[4], A. S. IVY[3];
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Med., Univ. of California, Irvine, Irvine, CA; [4]Dr. Richard A. Vladovic Harbor Teacher Preparation Acad., Wilmington, CA

Abstract: Introduction: Adverse childhood experiences such as neglect, abuse, and chronic stressors resulting from the COVID-19 pandemic, are increasingly recognized as having long-term impacts on pediatric cognitive health. There is an immediate need for interventions to offset the negative consequences of early-life adversity (ELA) on later-life cognition. Rodent models can be used to identify targetable mechanisms to offset the cognitive consequences of ELA. We developed a mouse model of early-life exercise (ELE) and discovered that ELE has lasting effects on hippocampal memory and synaptic plasticity (Ivy et al, *Sci Rep* 2020). We aim to identify memory-associated gene expression programs and their epigenetic regulators resulting from ELA and ELE, and apply ELE as a *post-hoc* strategy to gain mechanistic insights for therapeutic intervention after ELA. We hypothesize *that juvenile exercise introduced after ELA can prevent the negative consequences of ELA on hippocampal memory in adulthood*. Methods: We crossed Emx1$^{IRES\ cre}$ females with males harboring the NuTRAP cassette, a *loxP*-flanked Gt(ROSA)26Sor$^{tm1(CAG-birA,-mCherry/Rangap1,-EGFP/Rpl10a)Evdr}$ transgene, to generate the Emx-NuTRAP mouse. This allows for paired transcriptome and epigenome sequencing specifically from hippocampal neurons. During postnatal days (P) 2-9 Emx-NuTRAP mice are placed in limited bedding and nesting (LBN) cages to model ELA (Rice et al, *Endocrinology* 2008). ELE is then introduced during P21-41 to a subset of LBN mice. During young adulthood (P60+), mice underwent a battery of behavior tasks: 1) spatial y-maze, 2) object location memory task, 3) open field exploration (OFE), and 4) elevated plus maze (EPM). Middle-aged mice (8-10 month) will also be tested in the objects in updated locations (OUL) task (Wright et al, *Curr Protoc Neurosci* 2020). Once ELA-induced memory impairments are identified, RNA-Seq, ATAC-Seq, and CUT&Tag-Seq will be performed from isolated hippocampal neurons to evaluate molecular targets of memory reconsolidation after ELA and/or ELE. Results: Mouse running distances in the ELE paradigm were not significantly impacted by prior ELA exposure. ELA, with or without ELE, does not provoke differences in anxiety compared to controls in the OFE and EPM tasks, and all mice regardless of early-life exposure had similar performance in hippocampal short -and long-term memory tasks. Conclusion: We have established working models of LBN and ELE paradigms using Emx-NuTRAP mice. With pending OUL memory tasks and sequencing, we aim to identify exact memory impairments after ELA, and whether ELE can offset the consequences of ELA on memory function in middle-age.

Disclosures: N.E. Nelson: None. **B. Gomringer:** None. **S.D.A. Valientes:** None. **P. Abdoli:** None. **A. Bayat:** None. **C. Mandia:** None. **A.S. Ivy:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.05

Topic: F.04. Stress and the Brain

Support: NIH Grant 361711
T32DA007237

Title: Sex differences in the effects of early life stress on motivated behavior and transcriptional and post-translational regulation in the rat nucleus accumbens

Authors: *A. V. WILLIAMS[1], C. C. BAVLEY[2], R. KARBALAEI[1], E. ORDOÑES SANCHEZ[3], J. H. FLOWERS, II[1], K. S. COATES[3], A. INGRAM[1], B. A. GARCIA[5], D. M. WALKER[6], M. E. WIMMER[1], D. A. BANGASSER[4];
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Abstract: Early life stress (ELS) is a well-known risk factor for the development of several psychiatric diseases, including depression. Many studies on this topic have focused on cognition-related brain regions and behavior, so mechanisms underlying the impact of ELS on reward-related regions, such as the nucleus accumbens (NAc), and related reward-seeking and motivated behaviors are lacking. To begin to fill this gap, here we used the limited bedding and nesting model of ELS (LBN), which involves rearing pups in a limited resources environment, to study the impact of ELS on motivated behaviors for a natural reward, as well as on molecular mechanisms in the NAc.

Using Qiagen Ingenuity Pathway Analyses (IPA) we identified a sex difference in several predicted upstream regulators in a previously published NAc RNAseq dataset performed on LBN and control Long-Evans rats (group N's = 4-6), including BDNF and CREB1. Notably, CREB1 was significantly enriched as an upstream regulator in LBN females but not males. Additionally, IPA identified CREB signaling as a significantly enriched pathway in stressed females. Targets of this upstream regulator also differed between sexes. In females, CREB1 is a predicted regulator of 24 differentially expressed genes (DEG), of which 10 are activated and 14 are inhibited. In males, CREB1 is a predicted regulator of 6 DEGs, where 5 are activated and 1 is inhibited.

A genome-wide screen of histone posttranslational modifications (PTM) revealed differing patterns of LBN-driven PTMs in the NAc of males and females (group N's = 4-5). LBN increased the expression of 3 histone marks in males and decreased the expression of 1 histone mark in females. Acetylation of two of these marks (H4k12ac and Hk23ac) has been shown to be in part driven by CREB-binding protein (CBP). Additionally, previous work shows that acetylation of these histone marks in reward-related regions is altered by social defeat and chronic unpredictable stress in rats. This suggests CREB signaling and CBP-driven histone acetylation may be a driving force of stress phenotypes. Preliminary data from an ongoing study shows that LBN reduces motivation for a palatable food reward (sucrose pellets) in a progressive ratio operant tasks in non-food deprived adult male and female rats (group N's = 4-7; $p=0.025$). In upcoming studies we will assess the role epigenetic modifications, such as CBP-driven histone acetylation, may be playing in this LBN-driven depression-like phenotype.

Disclosures: A.V. Williams: None. **C.C. Bavley:** None. **R. Karbalaei:** None. **E. Ordoñes Sanchez:** None. **J.H. Flowers:** None. **K.S. Coates:** None. **A. Ingram:** None. **B.A. Garcia:** None. **D.M. Walker:** None. **M.E. Wimmer:** None. **D.A. Bangasser:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.06

Topic: F.04. Stress and the Brain

Support: NIH R00 Grant MH115096

Title: Sensitization of microcircuits by early-life stress in the nucleus accumbens

Authors: *J.-A. BALOUEK, C. A. MCLAIN, R. L. RASHFORD, T. ISLAM, C. J. PEÑA; Princeton Univ., Princeton, NJ

Abstract: In humans, early-life stress (ELS) heightens the risk of developing psychiatric disorders such as anxiety and depression. There is evidence that experiencing trauma early in life sensitizes individuals to future experiences of stress. However, little is known about the level at which this sensitization might be occurring in the brain. We hypothesized that ELS-induced stress sensitization may be evident at the level of neuronal microcircuits, such that cells activated by ELS might be more reactive to adult stress. We studied this by using a transgenic mouse line to tag and track experience-activated cells. We found that after going through an early-life stress paradigm, mice with an ELS history had an increased neuronal activation in the nucleus accumbens (NAc). We then postulated that the reactivation of ELS-responsive microcircuits in the NAc is a necessary component of the sensitized behavioral response to additional stress. To test this hypothesis, we performed stereotaxic surgery to target viral over-expression of *hM4Di*, an inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADD), in the NAc of male and female pups at 7 days old. This allowed for optimal expression of DREADD in experience-responsive cells in the NAc of mice during an ELS-specific time-sensitive period. During adult chronic social defeat stress (CSDS), we inhibited experience-responsive microcircuits with olanzapine, a ligand specific to inhibitory DREADDs, and studied the effect on subsequent depression- and anxiety-like behavior, in both male and female mice. We found interactions between inhibition of early-life-responsive cells and response to adult stress, which suggests that inhibitory DREADDs were expressed in distinct microcircuits in the NAc, responsive to either ELS or reward, and inhibition of these neuronal ensembles during CSDS reduced or enhanced the stress experience, respectively.

Disclosures: J. Balouek: None. **C.A. Mclain:** None. **R.L. Rashford:** None. **T. Islam:** None. **C.J. Peña:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.07

Topic: F.04. Stress and the Brain

Support: FONDECYT grant N° 1191274

Title: Type 1 corticotropin releasing factor receptor differentially modulates neurotransmitter levels in Nucleus Accumbens of juvenile versus adult rats

Authors: *J. ZEGERS[1], J. A. NOVOA[3], F. CALDERON[2], C. BLANLOT[2], H. E. YARUR[1], C. BASTIAS[2], K. GYSLING[4];
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Abstract: Juvenile rats exposed to social isolation have shown an increase of anxiety-like behavior and significant changes in Nucleus Accumbens (Nac) dopamine (DA) activity in their adulthood. Brain response to stress is mediated in part by the corticotropin releasing factor (CRF) system, composed by CRF and its two major receptors CRF-R1 and CRF-R2. In Nac shell, CRF induces anxiety-like behavior and changes in local DA balance. Nevertheless, the role of CRF receptors in controlling Nac neurotransmission is not fully understood. Previous results of our laboratory showed that the infusion of a CRF-R1 antagonist (CP-154,526) in Nac of juvenile rats increased DA levels in response to a depolarizing stimulus and decreased basal glutamate (Glut) levels. Nevertheless, the effect of CRF-R1 antagonism is not totally understood in adult rats. To evaluate the effect of a CRF-R1 antagonist (CP-154,526) we performed *in vivo* microdialysis in Nac of adult rats. We observed the opposite in Nac of adult rats compared to juvenile rats. The infusion of a CRF-R1 antagonist in Nac of adult rats decreased DA levels in response to a depolarizing stimulus and increased Glut levels in response to a depolarizing stimulus. Interestingly, we observed similar effects in Nac synaptosomes using an *ex vivo* approach. Furthermore, our results suggest that CRF-R1 controls glutamate levels through PKC signaling in Nac terminals. We propose that the function of Nac CRF-R1 receptors will be differentially modulated depending on the stage of life. Funded by FONDECYT grant N° 1191274

Disclosures: J. Zegers: None. **J.A. Novoa:** None. **F. Calderon:** None. **C. Blanlot:** None. **H.E. Yarur:** None. **C. Bastias:** None. **K. Gysling:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.08

Topic: F.04. Stress and the Brain

Support: NIH grant RO1 MH73136
NIH grant P50 MH096889
Donald Bren Foundation
Hewitt Foundation for Biomedical Research

Title: Single-cell transcriptomics of hypothalamic CRH-expressing neurons informs mechanisms by which early-life adversity sculpts enduring stress-responses.

Authors: *A. K. SHORT[1,2], C. WILCOX-THAI[3], A. L. PHAM[1], Y. CHEN[4], M. BIRNIE[1,2], J. L. BOLTON[2,1], A. MORTAZAVI[3], T. Z. BARAM[1,2];
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Abstract: Mental and cognitive health, as well as vulnerability to neuropsychiatric disorders, involve the interplay of genes with the environment, particularly during sensitive developmental periods. Early-life stress / adversity (ELA) promotes vulnerabilities to stress-related affective disorders, yet it is unknown how a transient ELA dictates life-long neuroendocrine and behavioral reactions to stress. The population of hypothalamic corticotropin-releasing hormone (CRH)-expressing neurons that regulate stress-responses is a promising candidate to mediate the enduring influences of ELA on stress-related behavioral and hormonal responses via enduring transcriptional and epigenetic mechanisms. Capitalizing on a well-characterized model of ELA, we examined here the resulting change in gene expression profiles in stress-sensitive CRH-neurons in the hypothalamic paraventricular nucleus (PVN) of male mice. Given the known heterogeneity of these neuronal populations, we employed single-cell RNA sequencing (RNA-seq) approaches. In pups, ELA-provoked changes clustered around expression of genes involved in neuronal differentiation, synapse formation, altered energy metabolism and cellular responses to stress and injury. The use of single-cell transcriptomics demonstrated that ELA-induced transcriptional changes took place in specific subtypes of CRH-cells, and primarily in select populations of glutamatergic CRH cells. Importantly, ELA-induced transcriptional reprogramming of hypothalamic CRH-expressing neurons heralded significant, enduring disruptions of both hormonal and behavioral responses to stress throughout life.

Disclosures: A.K. Short: None. **C. Wilcox-Thai:** None. **A.L. Pham:** None. **Y. Chen:** None. **M. Birnie:** None. **J.L. Bolton:** None. **A. Mortazavi:** None. **T.Z. Baram:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.09

Topic: F.04. Stress and the Brain

Support: NICHD grant HD091376

Title: Examining the synergistic effect of stress during puberty and pregnancy-related hormones on the PVN transcriptome

Authors: *K. N. GAUTIER[1], P. J. KANE[2], T. L. BALE[2], K. E. MORRISON[1];
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Abstract: Women who undergo adverse childhood experiences are at risk for developing lasting biological changes, including affective disturbances and stress dysregulation. Although we know this connection exists, the mechanisms underlying this relationship are unclear. We have previously shown that pubertal adversity is associated with a blunted glucocorticoid response within the hypothalamic-pituitary-adrenal (HPA) axis in both peripartum humans and mice. In mice, we have examined puberty-stress reprogramming in the paraventricular nucleus (PVN) of the hypothalamus, the brain region that initiates the HPA axis response. We found that pubertal stress led to an upregulation of immediate early genes (IEGs) in the PVN of adult, pregnant mice. IEGs are stimulus-dependent transcription factors that have many important downstream targets. Separately, we have shown that the pregnancy-associated hormone allopregnanolone is both necessary and sufficient to produce the blunted stress response phenotype in both pubertally-stressed adult female and male mice. Here, we assessed allopregnanolone as the potential mechanism underlying IEG expression changes associated with pubertal stress exposure. We hypothesized that administration of allopregnanolone would increase IEG expression in the PVN of pubertally stressed mice but not controls. Male and female mice underwent 14 days of chronic variable stress (CVS) beginning on postnatal (PN) day 21 using our previously established paradigm. CVS consisted of two stressors (tactile, olfactory, or auditory) per day. Control animals did not experience CVS. In adulthood (12 weeks old), all animals were given either allopregnanolone or vehicle (25% w/v HPβCD) treatment via two separate subcutaneous injections: 3 mg/kg of allopregnanolone 20 hours and 1 mg/kg 2 hours before brain collection. PVN micropunches were obtained from brains, RNA was isolated, and gene expression was measured using quantitative real-time PCR. If allopregnanolone is linked to increased IEG expression in the PVN, we expect to see that exogenous allopregnanolone increased each IEG of interest only in mice that experienced pubertal stress. Ongoing studies will address this question in a site-specific manner by delivering allopregnanolone directly into the PVN. These studies will further delineate the functional role for the increased IEGs in the pubertal stress-associated blunted HPA axis phenotype. Results from these studies will provide

novel insight into the mechanisms underlying female-relevant risk factors for stress dysregulation, a central endophenotype of affective disorders.

Disclosures: K.N. Gautier: None. **P.J. Kane:** None. **T.L. Bale:** None. **K.E. Morrison:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.10

Topic: F.04. Stress and the Brain

Support: NICHD Grant HD091376

Title: The impact of stress during puberty on the transcriptional state of the developing hypothalamus

Authors: *J. M. MENDOZA, K. N. GAUTIER, G. C. PIFER, R. B. GANDEE, S. L. HIGLEY, B. M. KAREM, C. A. KING, **K. E. MORRISON**;
Psychology, West Virginia Univ., Morgantown, WV

Abstract: Stress is a common occurrence, but excessive adversity during puberty can lead to negative outcomes in the brain. We have previously shown that stress during puberty leads to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis stress response, a key endophenotype of affective disorders, specifically during the peripartum window in both humans and mice. Using this translationally-relevant mouse model, we have additionally shown alterations to the transcriptome of the paraventricular nucleus of the hypothalamus (PVN), which initiates the HPA axis stress response. Specifically, pubertal stress resulted in an increase in six immediate early genes (IEGs) in the PVN of adult, pregnant mice in comparison to non-stressed pregnant mice, but in baseline conditions. IEG expression is canonically stimulus-dependent as they function to quickly produce necessary downstream signals. This baseline expression only in pubertally stressed females suggests potential aberrant transcription within the PVN. While these changes have been measured in adulthood, the exact timeframe for when the IEG transcriptional changes occur between the end of stress (early adolescence) and pregnancy in adulthood is unknown. We examined the developmental trajectory of IEG expression in the PVN immediately after pubertal stress. Male and female mice were exposed to chronic variable stress, or not, from PN 21-34. Twenty-four hours following the end of stress (PN35), brains were collected at baseline from non-stimulated animals. The PVN was collected by micropunch, RNA was isolated, and quantitative real-time PCR was performed to quantify relevant IEGs. If pubertal stress produces immediate reprogramming of the PVN, we expect to see increased IEG expression in pubertally stressed mice at PN 35 compared to controls. Alternatively, a lack of transcriptional changes immediately after stress will illustrate that pubertal stress has latent

effects only seen later in development or in combination with other dynamic life events, such as pregnancy. Ongoing studies will examine multiple time points following pubertal stress. Understanding the altered trajectory of the transcriptome in pubertally stressed mice will uncover whether pubertal stress leaves an immediate mark on the brain and will allow for potential opportunities for prevention of the adult phenotype. This translationally-relevant mouse model provides the opportunity to understand the molecular underpinnings of risk for stress dysregulation, a central endophenotype of affective disorders.

Disclosures: J.M. Mendoza: None. **K.N. Gautier:** None. **G.C. Pifer:** None. **R.B. Gandee:** None. **S.L. Higley:** None. **B.M. Karem:** None. **C.A. King:** None. **K.E. Morrison:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.11

Topic: F.04. Stress and the Brain

Support: NICHD grant HD091376

Title: The Impact of Pubertal Stress on Maternal Memory Formation

Authors: *G. C. PIFER, R. B. GANDEE, B. M. KAREM, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Stress early in life has been shown to have a negative impact on adaptive and efficacious parenting in humans. Previous studies have established that induced maternal behavior by exposure in virgin mice is enduring. We have previously shown that stress during puberty, an important period of brain development characterized by high neural plasticity, alters the hypothalamic-pituitary-adrenal (HPA) stress axis response in adult females only during pregnancy and postpartum. Specifically, pubertal adversity led to a blunted HPA response during pregnancy, which was associated with increased postnatal depression scores in humans. It is possible that pubertal stress creates a disorganization of maternal responsiveness and an increased vulnerability to affective disorders. We have not found major disruptions in pup retrieval behavior in pubertally stressed dams. However, in a maternal separation test during which dams were separated from pups by a mesh barrier, we observed that pubertally stressed dams were less likely to spend time at the barrier. The current study aims to build on these bodies of research by testing whether or not stress during puberty had an impact on the formation of maternal memory. We hypothesized that virgin females who experienced pubertal stress would have a deficit in the ability to form maternal memory in adulthood. Maternal memory is operationally defined as continued maternal responding toward pups during behavioral testing over time. Females were exposed to chronic variable stress (CVS) from postnatal day (PN) 21-

34, during which they received one stressor (olfactory, tactile, or auditory) per day for two hours each day. In adulthood (PN70), virgin females were tested for the ability to form maternal memory. This test was composed of two brief periods of exposure to unfamiliar pups, and then a subsequent pup retrieval task in a t-maze. The t-maze was repeated two weeks later with complete separation of females and pups in the interim. Brains were collected at PN84. Ongoing studies will examine a potential role for the medial preoptic area and paraventricular nucleus of the hypothalamus in any pubertal stress-induced alterations to the formation of maternal memory. The medial preoptic area is largely responsible for regulating maternal behavior, while the paraventricular nucleus initiates the HPA axis stress response. Therefore, stress during puberty could have a lifelong impact on continual parental responding and stimulation, which is supported by maternal memory. These results will provide novel insight into the impact of adversity during puberty on lifelong risk for altered maternal behavior.

Disclosures: G.C. Pifer: None. **R.B. Gandee:** None. **B.M. Karem:** None. **K.E. Morrison:** None.

Poster

P603. Early-Life Stress: Molecular Mechanisms, Cellular Effects, and Epigenetics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P603.12

Topic: F.04. Stress and the Brain

Support: NIH DK124727
NIH GM060507
NIH MD006988
Loma Linda University School of Medicine GRASP Seed Funds

Title: Psychosocial stress and obesogenic diet synergize to alter behavior and modulate FKBP5 methylation

Authors: *T. SIMON[1], P. ONTIVEROS-ANGEL[1], J. VEGA-TORRES[1], V. WILLIAMS[1], C. VILLALOBOS[1], F. SHARAFEDDIN[1], M. POMPILUS[4], M. FEBO[5], C. VIET[2], I. DELA PENA[3], J. FIGUEROA[1];
[1]Basic Sci., [2]Dent., [3]Pharmaceut. Sci., Loma Linda Univ., Loma Linda, CA; [4]Psychiatry, [5]Psychiatry Dept., Univ. of Florida, Gainesville, FL

Abstract: Background: Obesity and psychosocial stress are becoming more prevalent throughout the world and have been exacerbated by the Coronavirus 2019 outbreak. These environmental factors can shape neurodevelopmental trajectories, orienting neural circuits towards altered stress reactivity and emotional dysregulation. Adolescents that consume obesogenic diets enriched with saturated fats and carbohydrates are more susceptible to

dysregulated stress responses and behaviors. A pivotal biomarker associated with stress reactivity is *FKBP5*, which is a gene that encodes for the chaperone FK506 binding protein 51. This study investigated the potential involvement of *FKBP5* gene regulation in the neuropathological signatures associated with obesity and psychosocial stress. **Methods:** Adolescent Lewis rats (n = 56) were fed a Western-like diet (*WD*, 41% kcal from fat) or control diet (*CD*, 13% kcal from fat) beginning at postnatal day (PND) 21 and then subdivided into exposed and unexposed groups. The exposed groups endured a psychosocial stress model (PSS) that includes 30 consecutive days of social instability (PND 60 - 90) and two predator exposures (at PND 60 and 70). All groups underwent a battery of behavioral tests. Brain tissue was harvested at PND 107 and prepared for *FKBP5* gene methylation analyses, diffusion tensor imaging (DTI), neurohistology, and stress biomarker measurements.**Results:** Behavioral results reflected deficits in sociability and fear extinction along with an increase in anxiety-like behavior and food consumption in PSS/WD rats. DTI findings indicated a decrease in fractional anisotropy in the arcuate nucleus of the hypothalamus of rats exposed to PSS. We also found that PSS and the WD synergized to reduce *FKBP5* methylation in the hippocampus (approximately 8% hypomethylation upstream of the promoter and in multiple intronic and exonic regions). RNAscope confirmed altered *FKBP5* mRNA expression in the hippocampus of exposed to PSS/WD. We found higher fecal corticosterone levels in PSS/WD rats, supporting the observed *FKBP5* upregulation.**Conclusions:** Long-term consumption of an obesogenic diet during adolescence negatively affects behavior, stress responsiveness and increases the amount of *FKBP5* expression in the hippocampus. *FKBP5* hypomethylation may contribute to the amplified stress reactivity and obesity-related behaviors seen in those who have experienced childhood trauma. These results deepen our understanding of potential mechanisms linking early exposure to trauma and obesity.

Disclosures: T. Simon: None. **P. Ontiveros-Angel:** None. **J. Vega-Torres:** None. **V. Williams:** None. **C. Villalobos:** None. **F. Sharafeddin:** None. **M. Pompilus:** None. **M. Febo:** None. **C. Viet:** None. **I. dela Pena:** None. **J. Figueroa:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.01

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

Title: Role of C. elegans RAPGEF in Synapse Development at Neuromuscular Junction

Authors: *R. E. LAMB, S. J. CHERRA, III;
Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Guanine Exchange Factors (GEFs) are a family of proteins that activate GTPase signaling cascades. They accelerate the rate of GDP dissociation and subsequent GTP binding. The RAPGEF subfamily of GEFs are associated with multiple neurological disorders such as schizophrenia and myoclonic epilepsy. In murine models, disruption of RAPGEF6 function resulted in reduced anxiety behaviors, increased long term potentiation, but no changes in gross brain morphology. Although these studies suggest that RAPGEFs modulate synaptic function, the exact mechanism is currently unknown. To determine how RAPGEFs regulate synapses, we used the *Caenorhabditis elegans* neuromuscular junction. PXF-1 is the only *C. elegans* RAPGEF orthologue. We hypothesized that PXF-1 contributes to neural circuit function by promoting synapse development at the neuromuscular junction. To determine how PXF-1 modulates neural circuit function, we used aldicarb, an acetylcholinesterase inhibitor, to measure changes in motor circuit activity. Treatment with aldicarb induces paralysis that directly correlates with levels of cholinergic activity. We found that two independent mutant alleles of *pxf-1* caused the animals to become resistant to aldicarb in comparison to wild type animals. We then used fluorescently tagged synaptic proteins to investigate whether *pxf-1* mutants displayed alterations in synapse number or morphology. We detected no differences in the number of cholinergic synapses between wild type and *pxf-1* mutant animals, suggesting that PXF-1 does not regulate synapse formation. We then measured the area of presynaptic terminals in developing animals. We found that *pxf-1* mutant animals displayed a decrease in the size of puncta labeled by fluorescent synaptic vesicle and active zone proteins. To reveal the expression pattern of PXF-1, we used CRISPR/Cas9 genome editing to create a PXF-1::3xFLAG knock-in animal. In the nervous system, we detected PXF-1 in cell bodies of neurons and in processes near synapses. Based on our findings, PXF-1 may function in motor neurons to coordinate synaptic development and promote the function of neuromuscular junctions. Overall, our work provides a new insight into how RAPGEFs regulate nervous system function and how disruption of RAPGEF function may contribute to the development of certain neurological disorders.

Disclosures: R.E. Lamb: None. **S.J. Cherra:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.02

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

Support: NIH R01 #NS117588

Title: Establishing the cohort of early active zone proteins and their role in synaptic strength and maturation at the Drosophila neuromuscular junction

Authors: *E. J. GUSS, P. PUJOLS VÁZQUEZ, Y. AKBERGENOVA, J. LITTLETON;
Dept. of Biol. and Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Dysfunction in glutamatergic synaptic signaling can result in defects in learning and memory and contributes to neurodevelopmental disorders like intellectual disability and autism. Critical to normal formation of glutamatergic synapses is the development of active zones (AZs), specialized structures which cluster voltage-gated calcium (Ca^{2+}) channels (VGCCs), synaptic vesicles and the fusion machinery to ensure rapid Ca^{2+}-dependent vesicle fusion. Despite their importance, the molecular mechanisms that control AZ development and maturation at early stages of synaptogenesis are poorly understood. The *Drosophila melanogaster* larval neuromuscular junction (NMJ) provides an excellent genetically tractable system for understanding glutamatergic AZ development. Previous work in *Drosophila* determined that the AZ proteins Syd-1, Unc-13b and Liprin-alpha arrive early in AZ development, while the ELKS/CAST homolog Brp, Unc-13a, RIM Binding Protein and Ca^{2+} channels (Cacophony) arrive later in AZ development. Work in our lab has established that both developmental time and delivery of late AZ components correlate with release probability (P_r) and synaptic strength. In contrast, the full cohort of early AZ proteins is unknown, as well as how delivery of early AZ material correlates with synaptic strength and P_r. We are currently testing how manipulations of early AZ proteins alter structural and functional AZ maturation, as well as how they contribute to synaptic strength. Structural maturation is assessed by imaging and analyzing delivery of critical presynaptic proteins such as Cacophony and SNAREs, as well as formation of Glutamate receptor “rings,” a postsynaptic feature of maturing AZs. To identify novel early AZ seeding factors, we are also conducting CRISPR-based screens in single neurons, focusing on the role of lipid kinases and phosphatases as potential drivers of early AZ seeding. Together, these studies should provide important insights into early molecular events in AZ formation and their subsequent role in establishing synaptic strength at individual release sites.

Disclosures: E.J. Guss: None. **P. Pujols Vázquez:** None. **Y. Akbergenova:** None. **J. Littleton:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.03

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

Support: John S. Rogers Science Program

Title: Effect of Thrombospondin on NMJ Structure and Locomotion of Drosophila melanogaster

Authors: G. C. WOODS[1], K. COREY[1], S. M. LEE[1], I. MAXWELL[1], E. LOWENSTEIN[3], A. WOOTEN[3], L. ROTELLO[2], A. OSGOOD[4], D. MENDOZA ORTIZ[5], *N. A. VELAZQUEZ ULLOA[1];
[1]Biol., [2]Biochem. and Mol. Biol., Lewis & Clark Col., Portland, OR; [3]Oregon Hlth. & Sci. Univ., Portland, OR; [4]NIH/NHGRI, Bethesda, MD; [5]UNAM, Mexico City, Mexico

Abstract: Thrombospondin (TSP) is an extracellular matrix glycoprotein that plays a role in synaptogenesis at glutamatergic synapses in the mammalian brain. While there are 5 TSP genes in humans, there is a single homologous gene in *Drosophila melanogaster* (D-TSP) and there is conservation in the protein domains involved in TSP's function in synaptogenesis. It has not been investigated if D-TSP plays a role in synaptogenesis in *D. melanogaster*. Here we determined if D-TSP modulates synaptogenesis and locomotor behavior in the *D. melanogaster* third instar larval NMJ. We hypothesized that D-TSP would be necessary for normal NMJ formation and locomotor behavior. We used the GAL4-UAS system to knockdown D-TSP in neurons with two different RNAi lines and quantified features of the NMJ structure and locomotor behavior in larvae with normal or decreased D-TSP expression. All data come from at least two independent experiments and include the parental control lines and the experimental cross. Both RNAi experiments showed no significant survival differences between the parental lines and the cross. We took images of NMJs from muscle 4 of segments A2-4 in female third instar larvae from both RNAi crosses. In experiments with one of the RNAi lines, we manually counted the number of boutons, number of branches, number of branch points, and number of islands of each NMJ, and generated a complexity index comprising the number of branches, number of branch points, and number of islands. Our preliminary results suggest an increase in NMJ complexity when D-TSP is knocked down in neurons. We also calculated the polygon area for each NMJ, and found no differences in the D-TSP knockdowns. We are currently normalizing our morphological data by muscle area, and we are completing similar anatomical analyses with the second RNAi line. To investigate locomotor behavior, we took videos of male and female larvae moving in a gridded arena, and we performed a zones analysis that measures how far larvae moved from their point of origin. We did not determine sex differences in locomotor behavior. Larvae with the knockdown of D-TSP in both crosses with the RNAi lines show no changes in zones occupancy. We are in the process of validating the knockdowns of D-TSP by RT-qPCR. Next, we will explore a clumping phenotype seen in some NMJs, and measure other anatomical features. We will also quantify additional movement patterns, such as curvature, distance, and velocity, and we will analyze additional behaviors, such as head-turning and body contractions.

Disclosures: G.C. Woods: None. **K. Corey:** None. **S.M. Lee:** None. **I. Maxwell:** None. **E. Lowenstein:** None. **A. Wooten:** None. **L. Rotello:** None. **A. Osgood:** None. **D. Mendoza Ortiz:** None. **N.A. Velazquez Ulloa:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.04

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

Support: NIH Intramural Research Program Project 1ZIANS003137

Title: Temporal regulation of nicotinic acetylcholine receptor subunits supports central cholinergic synapse development in Drosophila

Authors: *J. ROSENTHAL[1,2], J. YIN[3], A. GRIGSBY-BROWN[4], J. LEI[4], A. SATHYAMURTHY[5], J. SHORT[4], C. LONG[4], E. SPILLMAN[6], C. SHENG[7], Q. YUAN[8]; [1]NIH, Bethesda, MD; [2]Dept. of Entomology, Univ. of Maryland, College Park, MD; [3]Natl. Inst. of Neurolog. Disorders and S, Bethesda, MD; [4]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [5]Ctr. for Neuroscience, Indian Inst. of Sci., Bangalore, India; [6]Dept. of Neurosciences, Univ. of California San Diego, San Diego, CA; [7]DMPU, NINDS, NIH, Bethesda, MD; [8]NIH/NINDS, Bethesda, MD

Abstract: The construction and maturation of the postsynaptic apparatus are crucial for synapse and dendrite development. The fundamental mechanisms underlying these processes are most often studied in glutamatergic central synapses in vertebrates. Whether the same principles apply to excitatory cholinergic synapses, such as those found in the insect central nervous system, is not known. To address this question, we investigated a group of projection neurons in the *Drosophila* larval visual system, the ventral lateral neurons (LNvs), and identified *Dα1* and *Dα6* as the main functional nicotinic acetylcholine receptor (nAchR) subunits in the larval LNvs. Using morphological analyses and calcium imaging studies, we demonstrated critical roles of these two subunits in supporting dendrite morphogenesis and synaptic transmission. Furthermore, our developmental expression profiling and endogenous tagging approaches identified distinct transcriptional controls over the two subunits resulting in the up-regulation of *Dα1, which is suppressed by elevated presynaptic activity*, and down-regulation of *Dα6* during larval development. Additional functional analyses of synapse formation and dendrite dynamics further revealed a close association between the temporal regulation of these two nAchR subunits and their sequential requirements during cholinergic synapse maturation. Finally, preliminary screens have identified candidate regulatory factors coordinating expression and postsynaptic activity of nAchRs, including transcription factors and molecular chaperones. Together, our studies identify temporal control of nAchR subunits as a core regulatory element for the development and plasticity of central cholinergic synapses, which likely involves both transcriptional and post-translational processes.

Disclosures: J. Rosenthal: None. **J. Yin:** None. **A. Grigsby-Brown:** None. **J. Lei:** None. **A. Sathyamurthy:** None. **J. Short:** None. **C. Long:** None. **E. Spillman:** None. **C. Sheng:** None. **Q. Yuan:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.05

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

Support: NIH 5 R21 NS109641-02

Title: Role of the cellular determinant Numb in NMJ size and function

Authors: T. SUKHANOVA[1], N. PRITCHETT[2], K. M. RENKEMEYER[2], B. A. BERKE[3], *H. KESHISHIAN[1];
[1]Molecular, Cellular, and Developmental Biol., Yale Univ., New Haven, CT; [3]Biol. Sci., [2]Truman State Univ., Kirksville, MO

Abstract: Drosophila motoneurons regulate synaptic plasticity using retrograde transsynaptic TGF-beta growth factor signaling. These signals can locally influence different levels of plasticity at distinct presynaptic branches of the same motoneuron. Potentially, asymmetrically localized molecular determinants might mediate this example of local branch plasticity. We examined Numb expression and function at the Drosophila larval NMJ. In 3rd instar larvae we observe Numb immunolabeling at synaptic boutons throughout the bodywall muscle fibers (mfs). The labeling was validated as Numb-specific by driving a presynaptic Numb RNAi knockdown to reduce levels of the antigen. Numb immunolabeling was observed in wild type larvae at type Ib and Is synaptic boutons. Numb loss of function phenotypes were tested using multiple presynaptic Numb-RNAi constructs. A significant reduction in bouton number was found at larval NMJs, scored at mfs 7&6, when compared to the control drivers (63.1 +/- 6.5 boutons, control Gal4 driver; 44.8 boutons +/- 2.6, Gal4-driven Numb RNAi knockdown, n=18 larvae). This is consistent with Numb's involvement in the presynaptic growth of the NMJ. An induced Geneswitch (GS) RNAi knockdown of presynaptic Numb partially suppressed activity-dependent plasticity in hyperactive K-channel (eag Sh) mutants (95.6 +/- 2.6 boutons, GS-uninduced Numb RNAi control; 75.7 +/- 4.7 boutons, GS-induced Numb knockdown, n=20 larvae). Electrophysiological studies point to a corresponding reduction of NMJ quantal content, consistent with the reduced bouton number. Ongoing studies will test genetic interactions with components of the TGF-beta signaling pathway. We hypothesize that Numb exerts a permissive role regulating NMJ size and plasticity, and could, through a localization to specific neural branches, influence presynaptic plasticity in a branch-specific fashion.

Disclosures: T. Sukhanova: None. **N. Pritchett:** None. **K.M. Renkemeyer:** None. **B.A. Berke:** None. **H. Keshishian:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.06

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

Support: NSF

Title: Postsynaptic neurotransmitter receptors regulate presynaptic neurotransmitter stability at the neuromuscular junction

Authors: *S. GODAVARTHI[1,2], N. C. SPITZER[1,2];
[1]Neurobio., Univ. of California San Diego, La Jolla, CA; [2]Kavli Inst. for Brain & Mind, Univ. of California San Diego, San Diego, CA

Abstract: The *Xenopus* neuromuscular junction (NMJ) is a well-studied cholinergic synapse that exhibits activity-dependent switching of the canonical presynaptic motor neuron (MN) neurotransmitter acetylcholine to glutamate, GABA, or glycine. These transmitter switches are accompanied by selection of the complementary postsynaptic receptors in myocytes, thus ensuring continued synaptic function and homeostatic plasticity. This raises a fundamental question: *Do postsynaptic receptors in myocytes influence expression of the cognate neurotransmitter in presynaptic neurons?* Although several membrane-permeant and secreted retrograde factors have been identified, it is not known if postsynaptic transmitter receptors participate in retrograde signaling. Using the *Xenopus* NMJ as a model system, we find that sparse *ectopic expression* of functional GABA receptors ($GABA_AR\alpha\beta\gamma$) in myocytes during development leads to expression of GABA in presynaptic cholinergic MNs. GABA expression is seen both in the local axon terminals innervating the $GABA_AR\alpha\beta\gamma$-expressing myocytes, and in cell bodies located in the spinal cord. Co-expression of the MN markers Hb1 and ChAT confirms that these cholinergic-GABAergic- neurons are indeed MNs. Further, the appearance of the GABA synthetic enzyme GAD67 and vesicular GABA transporter in these MNs suggests that MNs are capable of synthesizing and packaging GABA. Misexpression of a single $GABA_AR\alpha$ subunit, resulting in a non-functional GABAR that is not trafficked to the postsynaptic membrane, does not produce these changes. Using a GABA-insensitive mutant $GABA_AR\alpha\beta_{E179Q}\gamma$, we find that $GABA_AR$ channel activity is not necessary for presynaptic expression of GABA. Electrophysiological recordings from $GABA_AR\alpha\beta\gamma$ myocytes reveal that these NMJs exhibit GABAergic miniature endplate potentials (mEPPs) along with cholinergic mEPPs, showing that they use GABA functionally. Because these MNs normally express GABA transiently at earlier stages of development, exogenous $GABA_AR\alpha\beta\gamma$ seem likely to be stabilizing GABA expression. Loss-of-function studies blocking the endogenous acetylcholine receptors result in loss of ChAT in presynaptic MNs, recapitulating the role of receptor in stabilizing presynaptic NT expression. Our work indicates that postsynaptic receptors are not silent responders to changes in presynaptic neurotransmitter identity but actively shape presynaptic neurotransmitter stability. Such *receptor-mediated retrograde signalling* at the

synapse may be an acknowledgment signal for maintaining transmitter identity under normal conditions and play a role in synaptic remodelling and in neurological disorders.

Disclosures: S. Godavarthi: None. **N.C. Spitzer:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.07

Topic: B.06. Synaptic Transmission

Support: NIH Grant NS10560

Title: Tmem184b regulates neuronal excitability and locomotion in Drosophila larvae

Authors: *T. CHO[1], E. BEIGAITė[2], N. KLEIN[1], S. T. SWEENEY[2], M. BHATTACHARYA[1]; [1]Univ. of Arizona, Tuscon, AZ; [2]Univ. of York, York, United Kingdom

Abstract: The enigmatic Tmem184b seven-pass membrane protein was identified as a regulator of neuronal survival after injury and loss causes aberrant neuromuscular architecture. The mechanism through which Tmem184b affects neuromuscular synapses is currently unclear. Here we show that the *Drosophila* ortholog of human Tmem184b, Tmep, promotes efficient locomotion in *Drosophila* larvae. Tmep is expressed broadly, localizes to endosomes and is required for full viability. *Tmep* mutant larvae crawl less readily than controls, with stronger deficits in females. Using RNAi knockdown, we show that Tmep is required in motor neurons for proper larval locomotion. In recordings from body wall muscles, Tmep mutants show substantial hyperexcitability, with many post-synaptic potentials fired in response to a single stimulation. Neuromuscular junctions in Tmep mutants show modest structural deficits and endosomal aberrations. Locomotor defects can be rescued by knock-down of endoplasmic reticulum calcium channels, suggesting that excess cytosolic calcium may drive behavioral deficiencies. Our ongoing analysis of *Tmep* mutants aims characterise a novel link between endosomal function, calcium regulation and neuronal survival in the nervous system.

Disclosures: T. Cho: None. **E. Beigaitė:** None. **N. Klein:** None. **S.T. Sweeney:** None. **M. Bhattacharya:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.08

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

Support: HHMI GT 11432
NIH R01 NS 064263
NIH R25 GM 113686

Title: Transcriptional control of synapse elimination by a homeodomain transcription

Authors: *K. ALEXANDER[1], C. LAMBERT[1], M. DOITSIDOU[2], C. BENARD[3,1], A. WALKER[4], M. FRANCIS[1];
[1]Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA; [2]Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Univ. du Québec à Montréal,, Montréal, QC, Canada; [4]Mol. Med., Univ. of Massachusetts Med. Schoool, Worcester, MA

Abstract: Developmental remodeling of synaptic connectivity is an important process in the establishment of adult neural circuit organization. Central to this process is the elimination or pruning of juvenile synaptic connections. Defects in synapse elimination have been linked with several neuropsychiatric disorders, yet we have an incomplete understanding of the molecular mechanisms involved. I am investigating synaptic remodeling using the motor circuit of the nematode *Caenorhabditis elegans* as a model. A subset of GABAergic motor neurons, the dorsal D-class (DD) neurons, undergo an interesting form of remodeling where the axonal and dendritic domains switch during circuit development. This process requires eliminating juvenile synaptic connections and forming new synaptic contacts to establish the adult circuit. To identify key genes involved in synapse elimination, I carried out a forward genetic screen for mutants that fail to properly eliminate their juvenile synapses (visualized as clusters of GFP-tagged cholinergic receptors). From this screen, I isolated a mutation in the homeodomain transcription factor *dve-1*. In *dve-1* mutants, juvenile acetylcholine receptor clusters are not eliminated and instead persist into adulthood. Interestingly, the relocation of presynaptic components proceeds normally in *dve-1* mutants, such that, following remodeling, newly formed vesicle clusters identifying synaptic outputs of the mature circuit are interleaved with juvenile receptor clusters that would normally be eliminated. DVE-1 localizes to GABAergic motor neuron nuclei during remodeling, consistent with cell-autonomous DVE-1 transcriptional regulation of remodeling. To identify DVE-1 transcriptional targets that may be important for synapse elimination, we performed RNA-seq and identified several interesting candidates downregulated in juvenile *dve-1* mutants compared to wild type. Our findings begin to uncover a previously unknown transcriptional network for the control of developmental synapse elimination. We expect that our ongoing studies of transcriptional control of synapse elimination by DVE-1 will illuminate conserved genetic mechanisms controlling synapse removal during circuit development.

Disclosures: K. Alexander: None. **C. Lambert:** None. **M. Doitsidou:** None. **C. Benard:** None. **A. Walker:** None. **M. Francis:** None.

Poster

P026. Invertebrate Synapses

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P026.09

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

Support: SNSF; Ambizione PZ00P3_161448
SNSF; Assistant Professorship PP00P3_181036
Promotor Stiftung
Julius Klaus Stiftung

Title: The RNA-binding protein Musashi specifies synapse number and localization for individual axon branches via regulation of poly(A) tailing

Authors: *M. LANDÍNEZ-MACÍAS[1], W. QI[2], A. BRATUS-NEUENSCHWANDER[2], M. MUELLER[3], O. URWYLER[1];
[1]Dept. of Mol. Life Sci., [2]Functional Genomics Ctr. Zurich, [3]Univ. of Zurich, Zurich, Switzerland

Abstract: Central nervous system (CNS) circuit development critically relies on the correct specification of subcellular locations for synaptogenesis. Mutations in genes controlling synapse formation and organization are associated with neurodevelopmental and psychiatric disorders. Yet, our understanding of how synapse locations in neuronal processes are determined is very limited *in vivo*. Using single-cell labeling of mechanosensory neurons (MSN) in the *Drosophila* CNS, we here find that the evolutionary-conserved RNA-binding protein Musashi is required for determination of branch-specific numbers and subcellular locations of synapses. Remarkably, loss of Musashi leads to distinct synaptogenesis defects in individual CNS collateral branches of the same MSN axon. Musashi has additional functions in other CNS circuits. In olfactory receptor neurons, Musashi controls synaptic connectivity. Animals lacking *musashi* have mistargeting of olfactory receptor axons to a wrong glomerulus. We identify a type IIa receptor protein tyrosine phosphatase (Ptp69D), which localizes to future synaptic regions in developing axons, as a Musashi target. Loss of *ptp69D* causes similar synapse-patterning and synaptic connectivity defects as loss of *musashi*, and a genetic interaction between *musashi* and *ptp69D* supports their involvement in the same pathway. An RNA-editing approach ("TRIBE") reveals that Musashi binds to the 3'-untranslated region of *ptp69D* mRNA. Furthermore, a single-molecule sequencing approach shows that Musashi enhances *ptp69D* mRNA poly(A) tailing. Finally, *ptp69D* mRNA and protein levels are reduced in animals lacking *musashi*. We rationalize that *ptp69D* mRNAs with long poly (A) tails are more stable and translated more efficiently, and that loss of these mRNA molecules in *musashi* mutants reduces Ptp69D protein levels, thereby causing local synaptogenesis and synaptic connectivity defects. Together, our data reveal novel roles for Musashi in CNS wiring, and provide an entry point to the

understanding of how post-transcriptional mechanisms direct the establishment of axon branch-specific synaptogenesis.

Disclosures: M. Landínez-Macías: None. **W. Qi:** None. **A. Bratus-Neuenschwander:** None. **M. Mueller:** None. **O. Urwyler:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.01

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

Support: Chinese Science Council

Title: Axonal CB1 receptors mediate inhibitory bouton formation via cAMP increase and PKA

Authors: J. LIANG, ***C. J. WIERENGA**;
Utrecht Univ., Utrecht, Netherlands

Abstract: Experience-dependent formation and removal of synapses are essential throughout life. For instance, GABAergic synapses are removed to facilitate learning, and strong excitatory activity is accompanied by formation of inhibitory synapses to maintain coordination between excitation and inhibition. We recently discovered that active dendrites trigger the growth of inhibitory synapses via CB1 receptor-mediated endocannabinoid signaling, but the underlying mechanism remained unclear. Using two-photon microscopy to monitor the formation of individual inhibitory boutons in hippocampal organotypic slices from mice (both sexes), we found that CB1 receptor activation mediated the formation of inhibitory boutons and promoted their subsequent stabilization. Inhibitory bouton formation did not require neuronal activity and was independent of $G_{i/o}$ protein signaling, but was directly induced by elevating cAMP levels using forskolin and by activating G_s proteins using DREADDs. Blocking PKA activity prevented CB1 receptor-mediated inhibitory bouton formation. Our findings reveal that axonal CB1 receptors signal via unconventional downstream pathways and that inhibitory bouton formation is triggered by an increase in axonal cAMP levels. Our results demonstrate an unexpected role for axonal CB1 receptors in axon-specific, and context-dependent, inhibitory synapse formation.

Disclosures: J. Liang: None. **C.J. Wierenga:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.02

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

Support: 1R15 AG060461-(01)

Title: Examination of synaptic PSD-95 levels in the hippocampus of mature and developing NMDA receptor GluN2 chimera transgenic mice

Authors: *G. A. WILD[1], R. E. KEITH[2], T. C. DUMAS[1];
[1]Psychology, [2]George Mason Univ., Fairfax, VA

Abstract: In the hippocampus, N-methyl-D-aspartate receptors (NMDARs) are made up of two GluN1 subunits and two auxiliary GluN2 subunits (from GluN2A-D) and play central roles in synaptic plasticity and memory formation. During development, NMDAR composition shifts as expression of GluN2A increases in forebrain regions and synaptic content of GluN2A overtakes GluN2B. Previous research has shown that NMDAR integration into synapses is developmentally regulated by specific anchoring proteins in the postsynaptic density, including SAP102 and PSD95. In correlation to the auxiliary subunit shift, SAP102 is at its highest expression early in development, and decreases with age, whereas PSD95 is low at postnatal stages and increases during the course of the GluN2B to GluN2A switch. Knockdown of PSD95 prevents the GluN2B to GluN2A shift. However, the switch of subunits cannot be based solely on the change in PSD95 levels, as the scaffolding protein has been found to bind well to both GluN2A and GluN2B. Previous immunohistochemical research from our lab revealed increased expression of PSD95 in the hippocampus of young adult transgenic mice expressing GluN2 chimeras. PSD95 levels were increased in area CA1 and the dentate gyrus in “ABc” mice that express the N-terminus and transmembrane domains of GluN2A fused to the C-terminus of GluN2B. “BAc” mice, expressing N-terminus and transmembrane domains of GluN2B fused to the C-terminus of GluN2A, had PSD95 levels that were similar to wildtypes. Therefore, we sought to confirm the effect these GluN2 chimeras on PSD95 expression with more sensitive Western blots and extend the research to developmental animals. Preliminary results from young adult animals (P30-P60) demonstrate no differences in PSD95 levels between the different transgenic lines. Juvenile samples taken before and after the third postnatal week (P17-P19 and P22-P24) from both genotype lines are being quantified to determine any such differences. These findings will clarify any bidirectional interactions between NMDA receptors and PSD95 at hippocampal synapses yield greater insight into the complexity of synaptic maturation in the hippocampus.

Disclosures: G.A. Wild: None. **R.E. Keith:** None. **T.C. Dumas:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.03

Topic: B.06. Synaptic Transmission

Support: NIH Z01 ES100221

Title: Axonal inputs into mouse hippocampal area CA2 from the Dentate Gyrus

Authors: S. CHONG[1], D. RADZICKI[2], *S. M. DUDEK[1];
[1]Natl. Inst. of Env. Hlth. Sci., NIH, Research Triangle Park, NC; [2]Neurobio. Lab., NIEHS/NIH, Research Triangle Park, NC

Abstract: Hippocampal area CA2 is a molecularly and functionally distinct region of the hippocampus that had been classically defined as the area with large pyramidal neurons lacking input from the dentate gyrus and the thorny excrescences (TEs) characteristic of CA3 neurons. A modern definition of CA2, however, makes use of the expression of a number of genes that distinguish it from its neighboring CA3 and CA1 (RGS14 or PCP4, for example). Using this definition, it is evident that the axons originating from DG granule cells (DGGCs), forming what are known as mossy fiber (MF) synapses and contained in the *stratum lucidum* (SL), extend well into area CA2 to form excitatory synapses on the pyramidal neurons there. What is less known is how the presynaptic MF boutons (MFBs) and postsynaptic CA2 spines of these MF-CA2 synapses differ in comparison to the more highly characterized MF-CA3 synapses. Here we have implemented viral tracing, immunofluorescence, and confocal microscopy to compare the fine morphology of MFBs along the CA3-CA2 axis. Small-volume, targeted intracranial injections of AAV viruses into the dorsal hippocampus of wild-type C57 mice were used to express red or green fluorophores to label the pre- and postsynaptic elements of these synapses.Similar to previous findings, we observed many large ($\geq$ 6 μm^3) MFBs as well as the corresponding complex spines (thorny excrescences; TEs) on the CA3 apical dendrites, characteristic of the synaptic glomeruli typical of MF synapses. Previous work by Kohara, et al. demonstrated significant differences in pre- and postsynaptic complexity in the more distal regions of SL (i.e, smaller MFBs and lack of TEs in CA2). Similarly, we found fewer large MFBs in CA2. However, we found evidence of TEs in the SL of CA2, though much fewer in number than in CA3. Our observations indicate that TEs alone may not be suitable as a distinguishing feature differentiating CA3 from CA2 neurons. Additionally, we replicated Kohara's finding that dorsal DGGCs send long-range, longitudinal projections to the 'intermediate' CA2 but not to the corresponding CA3, suggesting that the axons are traversing through CA2 longitudinally. Within 100 μm of the distal-most SL, there was no significant difference in total MFB density in intermediate CA2 (0.0052 ± 0.0011 MFBs/μm^2) and dorsal CA2 (0.0042 ± 0.0012 MFBs/μm^2) in six sections from n= 3 mice (two tailed paired t-test: *p=0.57*). Ongoing 3D reconstruction and quantification of MFBs will help us to better understand potential differences in MF synaptic morphology along transverse and septotemporal axes and provide a basis for future functional studies.

Disclosures: S. Chong: None. **D. Radzicki:** None. **S.M. Dudek:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.04

Topic: B.06. Synaptic Transmission

Support: NSERC RGPIN-2015-05994
Research Manitoba New Investigator Grant
Research Manitoba studentship
Rady Faculty Health Sciences Scholarship
Autism Speaks Predoctoral Fellowship

Title: Lrrtm1 and lrrtm2 regulate synapse development and plasticity through distinct roles in developing and mature hippocampal circuits

Authors: *S. H. DHUME[1], S. CONNOR[3], F. MILLS[4], P. TARI[4], S. H. M. AU-YEUNG[5], B. KARIMI[2], S. OKU[5], R. T. ROPPONGI[2], H. KAWABE[6], S. X. BAMJI[5], Y. WANG[4], M. F. JACKSON[2], N. BROSE[7], A. CRAIG[8], T. J. SIDDIQUI[1];
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Abstract: Leucine rich repeat transmembrane neuronal proteins (LRRTMs) are a family of postsynaptic synapse organizing proteins that mediate excitatory synapse development through binding to presynaptic neurexins. LRRTM1 and LRRTM2 are highly expressed in the hippocampal CA1 and dentate gyrus regions However, their *in vivo* functions are poorly understood. To determine their role in synapse development and plasticity, we ablated *Lrrtm1/Lrrtm2* either globally from birth or locally in the CA1 and the dentate gyrus. We assessed the contribution of LRRTM1 and LRRTM2 to synapse numbers, synaptic composition and synaptic morphology using immunocytochemistry of brain sections, golgi staining and transmission electron microscopy. Loss of LRRTM1 and LRRTM2 from birth led to a marked reduction (by ~24%) of excitatory presynaptic inputs, assessed by vesicular glutamate transporter 1 (VGLUT1) puncta staining, to the CA1 dendritic compartments. In accord, ultrastructure studies showed that loss of LRRTM1 and LRRTM2 from birth reduced asymmetric synapse numbers by 27% when compared to wildtype controls. In contrast, VGLUT1 puncta density and synapse numbers were not altered in mature mice in which LRRTM1 and LRRTM2 were acutely deleted. Next, we assessed the role of LRRTM1 and LRRTM2 in synaptic function and plasticity using slice electrophysiology assays. We found that miniature excitatory postsynaptic current was significantly reduced (by ~30-35%) in both the developing and mature deletion models.

These deficits were accompanied by impaired long-term-potentiation and deficits in contextual fear memory. Our results suggest that LRRTM1 and LRRTM2 regulate excitatory synapse development, plasticity and behavior in a development-stage and context-dependent manner.

Disclosures: S.H. Dhume: None. **S. Connor:** None. **F. Mills:** None. **P. Tari:** None. **S.H.M. Au-Yeung:** None. **B. Karimi:** None. **S. Oku:** None. **R.T. Roppongi:** None. **H. Kawabe:** None. **S.X. Bamji:** None. **Y. Wang:** None. **M.F. Jackson:** None. **N. Brose:** None. **A. Craig:** None. **T.J. Siddiqui:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.05

Topic: B.06. Synaptic Transmission

Support: PhD Grant from the French Ministry of Higher Education and Research
INSERM
ANR PRosPECTIve ANR-18-CE16-0015-01
ANR THORNEX ANR-20-CE16-0028-01

Title: Loss of core PCP protein Vangl2 affects the morphofunctional development of the hippocampal mossy fiber synapse, resulting in working memory impairments

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Abstract: Deciphering how neurons acquire complex morphologies during development and how they modulate their core synaptic complexes to adapt to the environment during life and aging, is key to our understanding of how the nervous system is built and functions. A key brain structure in cognitive processes is the hippocampus, which is known for its role in episodic memory and context discrimination. The hippocampus is a highly connected region where we find a distinctive complex synaptic connection formed postnatally between the dentate gyrus (DG) and the CA3 sub-regions: the mossy fiber bouton / thorny excrescence synapse (MFB/TE). The morphogenesis of these large synapses critically depends on cytoskeleton remodelling, and variations of their morphology and size can impact their functional properties and alter behavioural processes. Amongst many regulators of cytoskeleton dynamics, the Wnt/PCP pathway is a conserved signalling pathway known to shape tissues during development via the

regulation of adhesion complexes and cytoskeleton. The PCP pathway plays a known role in prenatal brain development but its role in post-natal brain morphogenesis remains unclear. Here, we investigated the role of the core PCP protein Vangl2 in the post-natal development of the MFB/TE synapse, using a conditional *vangl2* knock-out (Vangl2f/f::EMX1-cre mice, henceforth called EMX1-Vangl2$^{-/-}$) mice. We show that Vangl2 is enriched postnatally in the DG and the CA3, during the period of development of the MFB/TE synapse. Using confocal and electron microscopy and 3D reconstruction, we show that the early loss of Vangl2 affects the size and complexity of the MFB/TE synapse in young mice and alter their structural plasticity in adults. Using electrophysiology, we also show that the basal properties of the MFB/TE synapses are altered by the early loss of Vangl2. Finally we show that the early deletion of *vangl2* impairs the performance of the adult animals in complex memory tasks. Altogether, our results shed a new light on the role of Vangl2-dependent PCP pathway in the brain, and more specifically provide evidence for the control and/or maintenance of circuits crucial for specific properties and components of relational/declarative memory.

Disclosures: N. Depret: None. **M. Gleizes:** None. **M. Moreau:** None. **G. Barthet:** None. **S. Dos-Santos Carvalho:** None. **A. Quiedeville:** None. **B.J. Robert:** None. **S. Al Abed:** None. **C. Mulle:** None. **A. Desmedt:** None. **C. Racca:** None. **A. Marighetto:** None. **M.E. Montcouquiol:** None. **N.A. Sans:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.06

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

Support: NIH Grant F32-NS106732
VA Merit Review Awards I01-BX002949
DOD W81XWH-18-1-0598
NIH Gran R21-NS102948
NIH Grant P30-NS061800

Title: Impact of mossy cell ablation on adult hippocampal neurogenesis

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Abstract: The neurogenic niche of the hippocampus allows for the incorporation of new dentate granule cells (DGCs) into the hippocampal circuit even in adulthood, and the proper integration of these new DGCs is critical for learning and memory. Disruption of adult-born DGC

proliferation and integration has been observed in animal models of traumatic brain injury, epilepsy, and stroke, often associated with hippocampal circuit changes such as hilar mossy cell loss. We hypothesized that hilar mossy cells contribute to early survival and integration of adult-born DGCs, and that the loss of mossy cells in disease could contribute to aberrant neurogenesis and integration of these new neurons. To test this idea, we selectively ablated hilar mossy cells via virus-mediated apoptosis in Crlr-Cre mice, and examined the proliferation, survival, and dendritic arborization and spine development of adult-born DGCs using BrdU and retroviral labeling. The removal of mossy cell inputs within the dentate gyrus did not impact proliferation or survival of adult-born DGCs. Ablation of mossy cells did accelerate dendritic outgrowth of adult-born DGCs, but did not affect the morphology of DGCs after maturation. Interestingly, despite the loss of axonal projections from mossy cells in the inner molecular layer, the dendritic spine density of 21 day-old DGCs was equivalent in the inner molecular layer and outer molecular layer with or without mossy cell innervation. Preliminary data suggests this may reflect reorganized excitatory inputs from the medial entorhinal cortex. Our results demonstrate that removal of hilar mossy cells accelerates outgrowth of adult-born DGCs, and that glutamatergic inputs in the molecular layer appear to compensate for the loss of mossy cell inputs. In ongoing work, we are examining the mechanisms linking mossy cell innervation to functional neurogenesis. Overall, our work suggests that loss of mossy cell inputs may drive reorganization of excitatory input within the dentate gyrus.

Disclosures: C.R. Butler: None. **G.L. Westbrook:** None. **E. Schnell:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.07

Topic: B.06. Synaptic Transmission

Support: NIH Grant MH107182

Title: Cyclic adenosine monophosphate signaling-mediated phosphorylation of diacylglycerol lipase-alpha regulates interaction with ankyrin-g and dendritic spine morphology

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Abstract: Diacylglycerol lipase α (DAGLα), a key biosynthetic enzyme for endogenous cannabinoid signaling, has emerged as a risk gene for several psychiatric disorders. However, its role in the regulation of dendritic spine plasticity is unclear. We found that DAGLα modulated dendritic spine size and density, but with different effects of changes in protein level and enzymatic activity, suggesting a 2-arachidonoylglycerol (2-AG)-dependent or independent

mechanism. The 2-AG-independent effect was mediated by the interaction of DAGLα with ankyrin-G, a multifunctional scaffold protein associated with psychiatric disorders. Using super-resolution microscopy, we observed coexistence in distinct nanodomains that correlated with spine size. In situ proximity ligation assay combined with structured illumination microscopy showed that upon forskolin treatment, DAGLα phosphorylation enhanced interaction with ankyrin-G in the spine, resulting in increased spine size and decreased DAGLα surface diffusion. Ankyrin-G conditional knockout mice showed a significant decrease in DAGLα-positive neurons in the forebrain. In mice, ankyrin-G was required for forskolin-dependent reversal of depression-related behavior. These data reveal novel synaptic signaling mechanisms and potential therapeutic approaches.

Disclosures: S. Yoon: None. **K. Myczek:** None. **P. Penzes:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.08

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

Support: Strengthening of Academic Bodies (PRODEP 2019-2021)

Title: Isocaloric low-protein diet during pregnancy decreases PUFA in breastmilk and synaptophysin area signal percentage in hippocampal CA1 and dentate gyrus male offspring

Authors: *M. NAVARRO MEZA[1], P. BELLO MEDINA[2], S. MARTÍN DEL CAMPO[3], E. DE LOS RÍOS ARELLANO[2], P. GARCÍA SOLANO[4], R. COBIÁN CERVANTES[4], F. SANTOYO TELLES[4], M. DÍAZ MUÑOZ[2];
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Abstract: Low-protein diet intake during pregnancy switch fatty acids concentration in breastmilk during lactation, these could be associated to alterations in the Nervous System in the postnatal stage in offspring. It is unknown about the effect of deficient protein intake during pregnancy on modifications of polyunsaturated fatty acids in breastmilk and changes of synaptogenesis in the hippocampus during neurodevelopmental in the neonatal stage. We analyzed if hypoprotein diet intake during pregnancy affects the percentage of polyunsaturated fatty acids in breastmilk and we studied in offspring synaptophysin area percentage in CA1 and dentate gyrus of the hippocampus. It was a longitudinal study in females rats exposed to a

hypoprotein isocaloric diet, during pregnancy and lactation 10 days. We used a control diet 18% protein and an experimental diet 6% protein. Were evaluated the feeding behavior and concentration of lipids and glucose in serum, fatty acids in breastmilk were determined by gas chromatography, and the synaptophysin area in pyramidal, oriens, and radiatum stratum of CA1 and granular, molecular, and lacunose molecular layer of the dentate gyrus of the hippocampus of the offspring by immunohistochemistry. We analyzed intragroup and intergroup. We showed that breastmilk from mothers exposed to hypoprotein diet was lower than control females (38%), fat content (50% lower) ($p<0.05$); polyunsaturated fatty acids were less (24%) linolenic acid concentration decreased 45% ($p<0.05$), and arachidonic and eicosanoic acid (100%) compared to breastmilk of mothers exposed to control diet ($p<0.05$). The synaptophysin area percentage in pyramidal and oriens stratum of CA1 in male offspring of mothers exposed to the hypoprotein diet decrease by 98% ($p <0.001$). Body weight decreased (5%) and concentration of high-density lipoproteins in serum in mother during lactation with hypoprotein diet. The glucose in serum decreased by 40-45% in the offspring of mothers exposed to a hypoprotein diet. These results suggest that hypoproteic diet intake during pregnancy and early lactation decrease the proportion of eicosanoic and arachidonic acid in breast milk, as well as the synaptophysin area percentage in pyramidal and oriens stratum of CA1 of male offspring in the neonatal stage, these results suggest that synaptogenesis has been altered in hippocampus in P10 neonates affecting the hippocampal network performance. Acknowledgment: Strengthening of Academic Bodies (PRODEP 2019-2021)

Disclosures: M. Navarro Meza: None. **P. Bello Medina:** None. **S. Martín Del Campo:** None. **E. De Los Ríos Arellano:** None. **P. García Solano:** None. **R. Cobián Cervantes:** None. **F. Santoyo Telles:** None. **M. Díaz Muñoz:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.09

Topic: B.06. Synaptic Transmission

Support: NIH Z01 ES100221

Title: Potential role of serotype and perineuronal nets in the preferential expression of adeno-associated virus-packaged genetic cargo in hippocampal CA2 neurons

Authors: *G. M. ALEXANDER, K. E. CARSTENS, B. GLOSS, S. R. LILLEY, B. S. SLAY, N. P. MARTIN, S. M. DUDEK;
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Abstract: A common approach to studying hippocampal circuits is through viral delivery of genetic material that encodes effector molecules and fluorophores. This is often accomplished through direct injection of adeno-associated viral (AAV) particles into the hippocampus, or via the bloodstream for some serotypes. In previous studies and in our own experiments, we have observed preferential expression of AAV-delivered cargo in CA2 compared to CA1 and CA3, even when not targeted to CA2. This curious observation led us to ask why CA2 neurons are preferentially targeted by AAVs. Because different AAV serotypes target different receptors, one possible mechanism by which differential AAV expression occurs is that CA2 neurons may preferentially express a receptor for specific serotypes of AAVs. Thus, we predicted that preferential expression of AAVs by CA2 neurons would vary with different serotypes, which target different receptors. Alternatively, we hypothesized that the preferential targeting of AAVs to CA2 is mediated by the dense expression of a specialized extracellular matrix, perineuronal nets (PNNs), which uniquely surround excitatory CA2 pyramidal neurons in addition to specific populations of inhibitory neurons throughout the brain.
To test the hypothesis that AAV expression in CA2 is dependent upon serotype, we injected AAVs of various serotypes, each coding for enhanced green fluorescent protein (eGFP) under the control of the human synapsin (hSyn) promotor, into the hippocampus of C57Bl/6 mice. Thus far, we have found that for each of multiple AAV serotypes injected intra-hippocampally, CA2 neurons preferentially express eGFP relative to CA1 and CA3 neurons. In addition, we found that eGFP was preferentially expressed in CA2 neurons after intravenous injection of the PHP.B serotype, which is known to cross the blood-brain barrier. To address the role of PNNs in AAV infection, we generated a conditional aggrecan gene (*ACAN*) knock-out (KO) mouse. Aggrecan is a primary component of PNNs detected around CA2 neurons as well as a class of interneurons. By pairing this “floxed” *ACAN* strain with our CA2-targeted *Amigo2* cre strain or parvalbumin (PV) neuron-targeted cre strain, we successfully deleted WFA-labeled PNNs from CA2 pyramidal neurons or from PV neurons, respectively. We injected CA2 *ACAN* KO mice with PHP.B-hSyn-eGFP intravenously and found that despite the absence of PNNs, CA2 neurons continued to express eGFP. These data do not support the hypotheses that preferential expression of AAVs by CA2 varies according to AAV serotype or that PNNs (or at least *ACAN*), is required for preferential expression in CA2 and point toward an alternative mechanism.

Disclosures: G.M. Alexander: None. **K.E. Carstens:** None. **B. Gloss:** None. **S.R. Lilley:** None. **B.S. Slay:** None. **N.P. Martin:** None. **S.M. Dudek:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.10

Topic: B.06. Synaptic Transmission

Support: MH097216
NS084959
R56NS094965
SNSF P2SKP3_161675

Title: Prrt2 is a driver of neuropsychiatric phenotypes in 16p11.2 microduplication mice

Authors: *M. FORREST[1], M. DOS SANTOS[1], N. PIGUEL[1], Y.-Z. WANG[2], N. HAWKINS[3], V. BAGCHI[1], L. DIONISIO[1], S. YOON[1], M. MARTIN-DE-SAAVEDRA[1], R. GAO[1], M. S. LEDOUX[4], J. A. KEARNEY[3], J. N. SAVAS[2], P. PENZES[1];
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Abstract: Copy number variants (CNVs) are a major class of genetic risk variants for neuropsychiatric disorders (NPDs), but the biological pathways involved in mediating risk are often unknown. The 16p11.2 microduplication (16p11.2$^{dup/+}$) is a highly pleiotropic CNV conferring risk of autism spectrum disorder, schizophrenia, intellectual disability, and epilepsy. Here, we use the 16p11.2$^{dup/+}$ mouse model to investigate the molecular, cellular and circuit basis for this shared NPD risk, and assess the contribution of individual genes to disease phenotypes. Using quantitative proteomics, we profiled neocortical membranes of 16p11.2$^{dup/+}$ mice to gain insight into pathophysiology. Membrane proteomics revealed an upregulation of synaptic proteins and a disruption of proteins associated to multiple NPDs. In particular, we identified a highly enriched network of synaptic and epilepsy-associated proteins in the mouse neocortex suggesting an alteration of cortical circuitry. Phenotypic characterization of 16p11.2$^{dup/+}$ mice uncovered cellular and circuit alterations including increased synaptic connectivity, hypersynchronous cortical circuitry, and increased seizure susceptibility. We hypothesized that a regulator of the proteomic changes in 16p11.2$^{dup/+}$ mice would have an important impact on pathophysiology. Using human co-expression data and interactome analysis, we find that PRRT2, a protein encoded within the microduplication, is a central hub in the dysregulated synaptic/epilepsy associated protein network. To evaluate the role of PRRT2 on disease phenotypes, we crossed 16p11.2$^{dup/+}$ mice with Prrt2$^{+/-}$ mice to correct PRRT2 copy number at the genomic level. Genetic correction of *Prrt2* reversed key neuropsychiatric associated with the 16p11.2 microduplication. Together, our work suggests that *Prrt2* is a major driver of disease biology in the 16p11.2 microduplication and that increased cortical connectivity and synchrony may be predisposing risk factors for diverse neuropsychiatric phenotypes.

Disclosures: M. Forrest: None. **M. Dos Santos:** None. **N. Piguel:** None. **Y. Wang:** None. **N. Hawkins:** None. **V. Bagchi:** None. **L. Dionisio:** None. **S. Yoon:** None. **M. Martin-de-Saavedra:** None. **R. Gao:** None. **M.S. LeDoux:** None. **J.A. Kearney:** None. **J.N. Savas:** None. **P. Penzes:** None.

Poster

P031. Synapse Maturation and Remodelling II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P031.11

Topic: B.06. Synaptic Transmission

Support: NIH grant 2R01MH097216

Title: Excitatory dysfunction drives synchrony deficits in a schizophrenia 16p11.2 duplication iPSC-derived neuron model

Authors: *E. PARNELL[1], L. CULOTTA[1], H. JALLOUL[1], *M. P. FORREST[1], M. DOS SANTOS[1], B. ECKMAN[1], D. D. LOIZZO[1], N. H. PIGUEL[1], D. J. TAI[2], M. E. TALKOWSKI[2], P. V. GEJMAN[3], J. DUAN[4], P. PENZES[1];
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Abstract: The 16p11.2 locus is a highly penetrant copy number variant (CNV) strongly linked to autism spectrum disorders and schizophrenia (SCZ). Whereas deletion at the 16p11.2 locus is a risk factor for numerous neurodevelopmental disorders, only duplications are linked to schizophrenia. There appears therefore, to be a unique role of 16p11.2 duplication in schizophrenia risk, suggesting a distinct etiological effect of 16p11.2 duplication. Using iPSC-derived induced cortical neurons from CRISPR-Cas9 isogenic 16p11.2 duplication lines and SCZ patient lines, penetrant 16p11.2 duplication phenotypes that may contribute to SCZ susceptibility were identified. Transcriptomic analyses identified deficits in neurite outgrowth, calcium handling and cell-cell junction gene expression. Consistent with this, iPSC from both isogenic and patient lines showed robust alterations in dendritic length, synaptic puncta and calcium handling. Moreover, these deficits converged on a penetrant alteration in network activity, revealed by network calcium imaging. Interestingly, network dynamics deficits were unchanged in the presence of iPSC-derived inhibitory neurons, supporting 16p11.2 duplication as a causative factor in excitatory cortical neuronal network dysfunction. These factors may contribute to excitatory neuron dysfunction in 16p11.2 duplication carriers early in development and promote cortical dysfunction and, thus, SCZ risk.

Disclosures: E. Parnell: None. **L. Culotta:** None. **H. Jalloul:** None. **M.P. Forrest:** None. **M. Dos Santos:** None. **B. Eckman:** None. **D.D. Loizzo:** None. **N.H. Piguel:** None. **D.J. Tai:** None. **M.E. Talkowski:** None. **P.V. Gejman:** None. **J. Duan:** None. **P. Penzes:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.01

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

Support: HFSP fellowship LT000479/2016-L
Simons Center for the Social Brain
Paul and Lilah Newton Brain Science Award,
NIH Grant R01MH16673
NIH Grant R01NS115543

Title: Brain-wide mapping the sequence of activity-dependent synaptic development

Authors: *J. XUE[1], A. BRAWNER[1], J. THOMPSON[1], T. YELHEKAR[1], Y. AHMED[2], K. NEWMASTER[3], Y. KIM[3], Y. LIN[1];
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Abstract: Synaptic development, which is highly influenced by neuronal activity, is a critical step during brain maturation that is required for the formation of functional neural circuits. This process is believed to be impaired in many neurodevelopmental disorders (NDDs), but it has been challenging to pinpoint when and where activity-dependent synaptic development is affected by the genetic and environmental factors in NDDs. Thus, there is an urgent need to design new tools and approaches to systematically monitor and examine activity-dependent synaptic development throughout the whole brain. To meet this challenge, we have constructed, for the first time, a longitudinal map of the developing mouse brain undergoing activity-dependent functional maturation during the early postnatal period. We generated a reporter mouse, Npas4-Cre, based on the activation of an immediate early gene (IEG) Npas4, which is selectively and robustly induced by neuronal activity and functionally important for synapse development. Crossing with a Cre-dependent reporter mouse, Ai75, neurons undergoing activity-dependent synaptic development, by activating Npas4 expression, are permanently labeled with nuclear-localized tdTomato (red). Combined with tissue clearing, whole-brain imaging, and computationally registering labeled neurons onto the newly developed developing mouse reference brains, we were able to monitor the sequence of synaptic maturation on the whole brain level. We observed a distinct sequence of brain regions undergoing synaptic maturation. For example, the neurons of sensory and motor-related regions (piriform cortex, somatosensory cortex, striatum et al.) are labeled earlier than regions associated with cognitive function (hippocampus). Using this approach, we were able to identify brain regions in which activity-dependent synaptic maturation was affected by developmental interventions that either enhanced (environmental enrichment, EE) or impaired brain development (NDDs). In addition to characterizing the synaptic development sequence, this approach allows us to carry out a multifaceted, detailed investigation of specific neuronal populations within developing neural circuits to uncover the underlying cellular and molecular mechanisms. We employed electrophysiology as well as transcriptomic experiments to identify molecular players important for synaptic development and behavior improvement in EE. The multipronged approach, enabled by the Npas4-Cre reporter mouse, has proven to be particularly powerful to identify targeting

regions and study genetic pathways impacted by developmental intervention, such as NDD mouse models.

Disclosures: J. Xue: None. **A. Brawner:** None. **J. Thompson:** None. **T. Yelhekar:** None. **Y. Ahmed:** None. **K. Newmaster:** None. **Y. Kim:** None. **Y. Lin:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.02

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

Support: NIH U01MH114825
NIH R35NS097305

Title: Emergence of synaptic modules during early cortical development

Authors: *L. ZHOU[1], A. BHADURI[2], Q. BI[1], S. WANG[1], A. KRIEGSTEIN[1];
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Abstract: Normal brain development requires electrical activity triggered by long-range inputs or local circuits, but little is known about the emergence of local cortical circuits during human brain development. We explore the physiological and morphological features of early intracortical connectivity in the developing human cortex during the second trimester, at the beginning of synaptogenesis. Single-cell Patch-seq demonstrates that electrophysiologically immature and morphologically simple cells in the cortical plate and marginal zone express genes for synaptic components. *Ex vivo* rabies tracing reveals the emergence of local synaptic modules in the cortical plate and subplate composed of immature excitatory neurons including migrating cells. Activity-dependent and independent spontaneous patterns of calcium activity can be observed in the synaptic modules. Furthermore, serotonin signaling regulates the formation of local synaptic modules through the activation of 5-HT2A receptors. Understanding the development of early intracortical circuits may shed light on the etiology of neurodevelopmental disorders and can inform *in vitro* models of human brain development.

Disclosures: L. Zhou: F. Consulting Fees (e.g., advisory boards); Neurona. **A. Bhaduri:** None. **Q. Bi:** None. **S. Wang:** None. **A. Kriegstein:** F. Consulting Fees (e.g., advisory boards); Neurona.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.03

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

Support: UVA Children's Hospital

Title: Development of GABAergic and glutamatergic synapses in the motor cortex

Authors: W. MATYSIK[1], J. KAPUR[2], J. BURNSED[1];
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Abstract: Synaptic development occurs when a synapse is formed between two neuron forms: presynaptic and postsynaptic terminals. There are no previous studies of the development of excitatory and inhibitory synapses in the motor cortex, which is considered a central region required for skilled voluntary movements, control, learning, and memory. The aim of our study was to compare synaptic development in brains of c57/Bl6 mice, between postnatal day (p)10 (neonatal) and p30 (young adults). We used immunohistochemistry for pre and postsynaptic markers such as glutamate receptor 1 (GluA1) and 2 (GluA2), vesicular glutamate transporter 1 (vGluT1), PDS-95, Bassoon, GABA (A) γ2, synaptophysin, and GAD65 to quantify and localize GABAergic and glutamatergic synapses. We used Western blot assays to quantify differences in protein production of synapses markers in the motor cortex. Lastly, we used the patch-clamp method to fill layer II/III neurons in the motor cortex with biocytin and then stained the slices with Streptavidin-AlexaFluor488 to assess the growth and length of dendrites. Western blot data shows that there was less production of pre and postsynaptic receptors in p10. The ratio of p10 to p30 for synaptophysin was 0.73 ± 0.03 (p=0.001), vGluT1 was 0.677 ± 0.02 (p=0.01). GluA2 and PSD-95 were not significantly different based on preliminary data. We observed an increase in pre and postsynaptic GABA and glutamate markers from p10 to p30 by a combination of immunohistochemical markers. We observed differences in the distribution within layers of the motor cortex with the use of GluA1 and GluA2 markers. We have also seen more intense staining in adults compared to neonates by looking at GluA2 and synaptophysin staining. These findings show differences in synaptic maturation in the motor cortex between neonatal and young adult mice under normal developmental conditions. Ongoing work focuses on evaluating surface expression of proteins and assessing the length and number of dendrites from p10 and p30 in layer II/III neurons in the motor cortex.

Disclosures: W. Matysik: None. **J. Kapur:** None. **J. Burnsed:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.04

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

Support: NIH Grant R01 NS045193
Fellowship by New Jersey Autism Center of Excellence (CAUT20AFP006)
Netherlands Organization for Scientific Research—Veni ZonMW, 91618112

Title: Comprehensive meta-analysis reveals a general cross-species timescale for neocortical synaptic development

Authors: *H.-J. BOELE[1], C. JUNG[1], L. A. LYNCH[1], D. PACUKU[1], M. TESTERMAN[2], S. GUARIGLIA[1], J. VERPEUT[4], **S. S.-H. WANG**[3];
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Abstract: Neocortical synaptic connections proliferate and are eliminated during development, a process that has been studied extensively over four decades. To place this work into a single scheme, we performed a meta-analysis on 16,000 data points extracted from 150 studies spanning 12 animal species, according to PRISMA guidelines and using SciBERT natural language processing to screen relevant literature. In altricial animals, synapse density started to increase soon after birth and peaked at around 1 gestational period postnatally, a pattern that persisted across neural subtypes, synapse type and location, and cortical layer. Brain regional differences were seen in humans and macaques. Synapse density declined over the next 4-5 gestational periods, with the exception of the human prefrontal cortex, which peaked at 10-15 gestational periods. Although evidence is still limited, autistic brains deviated from the overall pattern, with generally less spine pruning leading to higher synapse densities during adulthood. This first meta-analysis of synapse maturation will provide a scalable framework for understanding the mechanisms influencing brain development and a resource for identifying atypical neurodevelopment. (All data will be open-source, all data will be available online in a ShinyR dashboard.)

Disclosures: H. Boele: None. **C. Jung:** None. **L.A. Lynch:** None. **D. Pacuku:** None. **M. Testerman:** None. **S. Guariglia:** None. **J. Verpeut:** None. **S.S. Wang:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.05

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

Support: NSF Grant NeuroNex 1707352
NIH Grant U01NS099709
Grant W.M. Keck Foundation

Title: Developmental activation of pyramidal neurons differentially regulates cytoarchitecture and excitatory-inhibitory balance across distinct regions of the neocortex

Authors: *E. L. CRESPO[1], E. C. IKEFUAMA[2], N. J. COON[3], A. M. VAN LOO[4], G. FOLKERT[3], U. HOCHGESCHWENDER[5];
[1]Biochemistry, Cell. and Mol. Biol., [2]Neurosci., [4]Col. of Med., [3]Central Michigan Univ., Mount Pleasant, MI; [5]Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: Many psychiatric disorders are thought to have neurodevelopmental origins, stemming from malformation of neural circuits in early development. Early disruptions to cortical activity may underlie the later manifestation of behavioral changes typical of disorders such as autism. We carried out unbiased testing of the role of developmental over-excitation in altering adult behavior and circuit dynamics. We took advantage of Bioluminescent Optogenetics (BL-OG), where light stimulation of an optogenetic element is achieved either through bioluminescence emitted from a tethered luciferase upon application of a chemical substrate or through application of physical light via fiber optics. Mice conditionally expressing LMO3, a fusion of sbGLuc and VChR1, were crossed with Emx1-Cre transgenic mice, thus limiting expression of LMO3 to cortical pyramidal neurons. By delivering the luciferin CTZ intraperitoneally during post-natal days 4-14, over-excitation was induced chemogenetically in the cortical pyramidal neurons of developing mouse pups. During adulthood, mice were tested behaviorally, assessed for electrophysiological changes in circuit dynamics by standard optogenetic interrogation, and probed for changes in interneuron numbers. While Emx1-positive neurons were hyperexcited during postnatal development across the cortex, behavioral, electrophysiological, and morphological effects manifested differently in distinct areas.

Disclosures: E.L. Crespo: None. **E.C. Ikefuama:** None. **N.J. Coon:** None. **A.M. Van Loo:** None. **G. Folkert:** None. **U. Hochgeschwender:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.06

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

Title: Conserved molecular signatures for probing subclass-specific circuit motifs in the adult neocortex

Authors: *A. J. MOUSSA, J. C. WESTER;
Neurosci., The Ohio State Univ., Columbus, OH

Abstract: A prevailing challenge in neurobiology is understanding how diverse neuronal cell types integrate into circuits with emergent functions. We mined the Allen Institute for Brain Science's single-cell RNA-sequencing database of adult mouse cortical neurons to identify subclass-specific molecular signatures of circuit architecture across the cortex. We analyzed differential expression (DE) of cellular adhesion molecules (CAMs) and their regulatory genes among major subclasses of GABAergic (inhibitory) and glutamatergic (excitatory) neurons in two brain regions: the anterior lateral motor cortex (ALM) and the primary visual cortex (VISp). We hypothesized that differential expression of CAMs among major neuronal subclasses is conserved between these two functionally distinct cortices. We used the R package Seurat to determine differential gene expression and filtered the results using the gene ontology (GO) software PANTHER 16.0 to classify DE genes by selected terms: "Cell-Cell Adhesion", "Regulation of Cell-Cell Adhesion", and "Regulation of Trans-Synaptic Signaling." Downstream bioinformatic analysis was conducted to identify unique and conserved DE patterns among subclasses both within and between ALM and VISp. Our analysis indicates that both glutamatergic and GABAergic subclasses display region-independent conservation of gene expression associated with synaptic connectivity, suggesting cell type-specific wiring principles. Thus, subclass identity likely plays a greater role than cortical region in determining local circuit architecture. Finally, we used ClinVar Miner to focus on genes associated with neurological disorders identified through clinical testing. ClinVar review uncovered that 14% of the DE CAM-related genes are relevant to neurological disorders, with many defining conserved regulatory relationships across subclasses and cortices in both glutamatergic and GABAergic neurons. These findings thereby associate disease-relevant genes with specific neuron subclasses to provide candidate mechanisms, targeted to these subclasses, for investigating aberrant circuit architecture in future studies.

Disclosures: A.J. Moussa: None. **J.C. Wester:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.07

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

Support: NIH Grant R211EY032381

Title: Emergence of coordinated network activity in the developing mouse parietal cortex.

Authors: *L. MA[1,2], J. HOFFMAN[4], J. N. GELINAS[2,1,3];
[1]Biomed. Engin., Columbia Univ., New York, NY; [2]Inst. for Genomic Med., [3]Neurol., Columbia Univ. Med. Ctr., New York, NY; [4]Univ. of Alberta, Edmondon, AB, Canada

Abstract: Mature rodent parietal cortex (PPC) connects with a multitude of cortical regions and subcortical structures, playing an essential role in brain functions such as association and navigation. Such complex functions require intercortical communication, which is often facilitated by neural oscillations. However, precisely coordinated oscillatory activity enabling cognition is not an innate property of the underlying neural networks. To investigate how such patterns emerge, we performed *in vivo* electrophysiology on mouse pups between postnatal day 5 and postnatal day 14. Using flexible, conformable, and high-density electrode arrays with high spatiotemporal resolution and large cortical coverage, we recorded local field potentials (LFP) and action potentials from dorsal cortical brain regions of mouse pups as they cycle through normal sleep states. We identified electrodes recording from primary sensory (S1), primary visual (V1), and parietal cortex using vesicular glutamate transporter 2 (VGluT2) immunohistochemistry. Although spindle burst oscillations are a dominant pattern in early development of S1 and V1, there was a paucity of such activity in PPC. Independent PPC oscillatory activity could be acquired starting in the second postnatal week, with distinct frequency and waveform characteristics. We also found evidence of intercortical comodulation of oscillatory activity during this epoch. These results suggest that PPC neural activity is developmentally regulated, with a maturation pattern distinct from neighboring primary sensorimotor cortices and potentially linked to emergence of higher cognitive processes.

Disclosures: L. Ma: None. **J. Hoffman:** None. **J.N. Gelinas:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.08

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

Support: Wellcome Trust 215199/Z/19/Z
BBSRC project grant BB/P003796/1
MRC grant MR/T033320/1

Title: Networks and function of somatostatin-expressing interneurons across primary sensory areas of the mouse neocortex during early postnatal development

Authors: *F. GHEZZI, L. J. BARUCHIN, N. T. HA, S. J. B. BUTT;
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Abstract: GABAergic interneurons (INs) play an essential role during the development of mammalian neocortical network. Somatostatin-expressing (SST+) INs are optimally suited to provide the main source of GABAergic signalling during postnatal development as they integrate early into the cortical network. In mouse primary somatosensory barrel cortex (S1BF), layer (L)5b SST+ INs establish a transient, translaminar, reciprocal circuit with L4 spiny stellate neurons during the critical period for plasticity (postnatal day (P)5-8). This network plays a role in timing the emergence of sensory-driven, feed-forward inhibitory architecture in L4. However, whether this transient network represents a common strategy employed by other brain areas during early postnatal development remains to be investigated. Through *in vitro* electrophysiology and circuit mapping techniques, we studied GABAergic networks of the mouse primary visual cortex (V1) during the first 3 weeks of development. Our results show that the transient L5b-L4 circuit found in S1BF is not present in V1 through postnatal life, rather thalamocortical-recipient L4 pyramidal neurons receive local GABAergic input from within L4. Furthermore, SST+ INs in infragranular layers form a distinct translaminar network with L2/3 pyramidal neurons during the first postnatal week of development. We next explored the contribution of these INs to emergent somatosensory and visual perception *in vivo*. We expressed Channelrhodospin-2 in either *SST* or *Nkx2-1* Cre driver lines and used optogenetic spike-tagging to identify the contribution of SST+ INs and parvalbumin-expressing (PV+) INs to both spontaneous activity and sensory-evoked cortical responses. Our results reveal that SST+ interneurons are engaged by sensory stimuli prior to the onset of active perception in both sensory areas. However, the layer location of sensory-responsive SST+ INs differs between S1BF and V1, in line with our *in vitro* circuit mapping. As such we demonstrate that local GABAergic networks readily differ across primary sensory areas from early development postnatal life. This suggests that genetic mutations that cause an impact on the network integration of GABAergic interneuron will result in circuit dysfunction in an area-dependent manner with selective consequences for emergent function. In conclusion, our study highlights that general principles for synaptic integration and circuit formation cannot be extrapolated across cortical areas with consequences for our understanding of neurodevelopmental psychiatric disorders.

Disclosures: F. Ghezzi: None. **L.J. Baruchin:** None. **N.T. Ha:** None. **S.J.B. Butt:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.09

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

Support: NIH Grant R01MH116176

Title: Whole Brain Mapping of Developmental Neural Activity in Oxytocin Receptor Deficient Mice

Authors: *K. NEWMASTER[1], Y. KIM[2];
[1]Pennstate Col. of Med., Middletown, PA; [2]Col. of Medicine, Penn State Univ., Hershey, PA

Abstract: Neural activity during early postnatal development plays an important role in establishing neuronal wiring patterns. Oxytocin regulates neuronal activity and maturation via the oxytocin receptor (OTR). In our previous mapping of OTR expressing cells across the whole mouse brain during postnatal development, we found that OTR has specific spatiotemporal expression patterns, and it is expressed in both glutamatergic and GABAergic cell types. However, it remains unclear how oxytocin signaling in different cell types is affecting neural activity in the developing brain. Here we investigate neural activity throughout the entire mouse brain on postnatal day (p)7 and p14 in cell type specific OTR knockout (KO) mice which were generated using either Gad2-Cre or vGlut2-Cre to mediate conditional OTR KO. To visualize neural activity in developing brains, we used IDisco to label the immediate early gene, cFos a protein marker for neural activity. These brains were imaged using light sheet fluorescent microscopy and detected signals were quantified with age matched reference brains. cFos quantification in normal mice revealed significant activation of the cortical subplate (SP) and paraventricular thalamus (PVT) at p7, while regions involved in reward processing and stress such as the ventral tegmental area (VTA) show increased cFos-positive cells at p14. Interestingly, lack of OTR in glutamatergic cells lead to a decreased cFos levels (20%) in the PVT, VTA, and other reward related regions at p14. In contrast, lack of OTR in GABAergic cells increaser p14 cFos levels in stress related regions such as the central amygdala (CEA) and bed nucleus stria terminalis (BST) (20%). These patterns suggest that Oxytocin modulates the development of reward and aversion processing during the second postnatal week.

Disclosures: K. Newmaster: None. **Y. Kim:** None.

Poster

P035. Neural Circuit Maturation and Remodelling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P035.10

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

Support: DFG grant KI 1816/6-1
DFG grant KI 1816/7-1
DFG grant HO 2156/5-1
DFG grant HO 2156/6-1
DFG Research Unit 3004

DFG Priority Program 1665
DFG CRC Transregio 166

Title: A transient bursting period during the emergence of synaptic inhibition in the developing hippocampus in vivo

Authors: *J. GRAF[1], V. RAHMATI[2,3,1], M. MAJOROS[1], O. W. WITTE[1], C. GEIS[2,1], S. KIEBEL[3], K. HOLTHOFF[1], K. KIRMSE[4,1];
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Abstract: Immature neural circuits are characterized by spontaneously occurring synchronized network events that involve large neuronal populations. Within the first postnatal month in rodents, there is a transition to desynchronized and continuous activity. What are the cellular dynamics and intrinsic biophysical mechanisms underlying neuronal synchrony in the intact developing brain? To address this question, we use two-photon Ca^{2+} imaging to analyze neuronal network activity in the developing mouse hippocampal area CA1 at single-cellular resolution *in vivo*. Strikingly, we find an intermediate network bursting period in the second postnatal week, i.e. after the developmental emergence of effective synaptic inhibition. At this time, pyramidal neurons fire in an almost random manner, and pairwise neuronal correlations are low. We reveal that enhanced network synchrony reflects an increased functional coupling of the activity of individual neurons to that of the local population. Dynamic systems modeling identifies network bi-stability as a potential regime underlying network burstiness at this age. We further show that network bursts depend on proper input timing and strength, pointing to the emergence of the network's capability of input selectivity. We thus propose that CA1 network bursts underlie extensive input-discrimination learning prior to the onset of environmental exploration.

Disclosures: J. Graf: None. **V. Rahmati:** None. **M. Majoros:** None. **O.W. Witte:** None. **C. Geis:** None. **S. Kiebel:** None. **K. Holthoff:** None. **K. Kirmse:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.01

Topic: A.07. Developmental Disorders

Support: NIH/NICHD P50 HD103573

Title: Effects of prenatal stress in a genetic mouse model of autism

Authors: K. M. HARPER, J. A. JIMENEZ, M. J. ZYLKA, *S. S. MOY;
Univ. of North Carolina at Chapel Hill Sch. of Med., Chapel Hill, NC

Abstract: Autism spectrum disorders have complex etiology, which likely includes interactions between multiple genes and/or environmental factors. *CHD8* is a high confidence autism gene, but ASD-like phenotypes in *Chd8* heterozygous (HET) or mutant mouse models have varied greatly and are often mild. This study investigated whether prenatal exposure to maternal stress would increase the emergence and severity of behavioral phenotypes in *Chd8* HET mice. Subjects were offspring of C57BL/6J wild-type dams bred to *Chd8* HET males. Dams in the stress condition underwent a series of behavioral tests, including acoustic startle and conditioned fear, across gestational days 10 to 15. Control dams received only routine husbandry care. Offspring were weaned at 22 days in age and evaluated for ASD-like and other phenotypes, starting at 4 weeks in age. The testing battery included assays for anxiety-like and depression-like behavior, activity, social approach, olfaction, and conditioned fear. Preliminary results indicate significant effects of both prenatal stress and genotype in a subset of tests, with different behavioral profiles observed in males and females. Overall, this study extends the characterization of the *Chd8* HET mouse as a gene x environment model for ASD-like phenotypes.

Disclosures: K.M. Harper: None. **J.A. Jimenez:** None. **M.J. Zylka:** None. **S.S. Moy:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.02

Topic: A.07. Developmental Disorders

Title: Microrna as a potential biomarker in autism: prenatal stress and SERT gene interaction model

Authors: *T. WOO[1], C. KING[2], C. BLOOMER[3], N. KIBIRYEVA[4], Z. TALEBIZADEH[5], D. Q. BEVERSDORF[6,1];
[1]Interdisciplinary Neurosci. Program, [2]Biol. Sci., Univ. of Missouri, Columbia, MO; [3]Mol. and Integrative Physiol., Univ. of Kansas Med. Ctr., Kansas City, KS; [4]Ward Family Heart Ctr., Children's Mercy Hosp., Kansas City, MO; [5]Children's Mercy Hosp. and Univ. of Missouri-Kansas City Sch. of Med., Kansas City, MO; [6]Dept Radiol, Neurol, Psychol Sci, DGS of INP, Univ. of Missouri Columbia, Columbia, MO

Abstract: Prenatal stress is critical in neurodevelopment and increases risk for autism spectrum disorder (ASD), particularly in those genetically susceptible to stress. In animal models, we demonstrated that aberrant social behavior was observed in offspring of heterozygous serotonin

transporter knock-out (SERT-het) mice exposed to stress during pregnancy, and restricted social interaction was associated with epigenetic changes in the embryonic brains. Two independent clinical studies have shown that the higher incidence of ASD in prenatally stress-exposed mothers is associated with maternal presence of the SERT short-allele (deletion in promoter region). Recently, we have reported differential expression (DE) of microRNAs (miRNAs) in blood samples of prenatally stress-exposed mothers carrying short allele who have children with ASD, which may serve as a potential blood biomarker of the gene x environment (G x E) interaction in ASD. To further investigate the role of miRNAs as a potential biomarker during pregnancy in this G x E interaction model, we profiled the miRNA expression in blood from dams in the mouse model of SERT-het/stress dams at embryonic day 21 (E21) and postnatal day 60 (PD60) and compared with that of wild-type mice at the same time window. More than three thousand mature miRNAs were examined, and the ANOVA analysis detected several DE miRNAs. However, there was no common DE miRNAs between E21 and PD60, which suggested a transition in miRNA profiles in SERT-het/stress dams after delivery. One of upregulated miRNAs (miR-149-3p) at PD60 and two of upregulated miRNAs (miR-328-5p and miR-762) at E21 were also found in the DE miRNAs of human samples from our previous study. These miRNAs could be biomarker candidates for G x E interactions in ASD, and are highly dynamic over time. Our study provides evidence for epigenetic alterations associated with the gene x environmental interaction which we hope will lead to better understanding of the causes and treatments of ASD, resulting from prenatal stress exposure in genetically stress susceptible individuals.

Disclosures: T. Woo: None. **C. King:** None. **C. Bloomer:** None. **N. Kibiryeva:** None. **Z. Talebizadeh:** None. **D.Q. Beversdorf:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.03

Topic: A.07. Developmental Disorders

Support: Partnership for Pediatric Epilepsy Research
Autism Speaks
Norton Children's Hospital grant
NIH P30ES030283 Pilot grant

Title: Increased oxidative injury and blood brain barrier disruption in the brain of two murine models of autism spectrum disorder.

Authors: *E. GOZAL[1], R. JAGADAPILLAI[1], T. CHU[1], J. CAI[1], Z. LI[2], G. N. BARNES[3]; [1]Dept of Pediatrics PRI, Univ. of Louisville, Louisville, KY; [2]Neurol., Vanderbilt Univ., Nashville, TN; [3]Dept Neurol, Univ. of Louisville Sch. of Med., Louisville, KY

Abstract: Immune dysregulation, oxidative stress and neuroinflammation with glial activation have been proposed to play a role in the pathogenesis of Autism Spectrum Disorder (ASD). A study of post-mortem human ASD brains showed altered expression of genes associated with blood brain barrier (BBB) integrity and increased neuroinflammation. However, few studies exist of the involvement and the mechanisms of BBB disruption in autism animal models, such as BTBR or neuropilin-2 (NRP2)/Semaphorin 3F (Sema 3F) KO mice. BTBR mice, an inbred mouse model of ASD, exhibit a neuroinflammatory profile that correlates with some of their repetitive behavior, characteristic of ASD. We have previously described a neuropilin-2 (NRP2) knockout as a novel murine genetic model of autism and epilepsy. GABAergic interneurons-selective deletion of the NRP2 ligand, Sema 3F, is associated with decreased interneurons numbers, ASD behavioral symptoms, and showed microglial activation and increased inflammatory and oxidative markers in several brain regions that could contribute to BBB dysfunction. As neuroinflammation is common to both BTBR and Sema3F KO ASD models, we investigated whether both models exhibit oxidative markers and BBB disruption and could provide reliable models to study how these characteristics may lead to the disease in humans.BTBR, C57BL/6 control mice, Sema 3F KO and WT mice were sacrificed and perfused for histological analysis. DHE staining showed increased superoxide formation in the cortex, hippocampus and amygdala of BTBR and of Sema 3F KO mice, compared to their respective controls, attesting to increased oxidative stress in various ASD mice brain areas relevant to autism. Immunostaining for albumin showed that while perfusion removed most of the vascular albumin from control mice tissue, significant albumin remained in the tissue of Sema 3F KO and BTBR mice, suggesting vascular leakage. Intracardiac administration of fluorescently labeled Dextran tetramethylrodamine 10kD to BTBR or C57BL/6 mice, prior to sacrifice, showed some extravasation in the cortex of BTBR, but not of C57BL/6, confirming our data. Additionally, zonula occludens protein 1 (ZO1), a tight junction protein significantly decreased in BTBR mice brains. In conclusion, 2 different murine ASD models, the inbred BTBR polygenic model and the monogenic Sema3F KO, share enhanced neuroinflammation, oxidative injury and BBB disruption markers, also reported in humans, and that could directly contribute to autistic behavior impairment.

Disclosures: E. Gozal: None. **R. Jagadapillai:** None. **T. Chu:** None. **J. Cai:** None. **Z. Li:** None. **G.N. Barnes:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.04

Topic: A.07. Developmental Disorders

Support: NIMH Grant R01 MH121674
NARSAD Young Investigator Award (27818)

Title: Taok2 mediates er-microtubule tethering during neuronal development

Authors: *S. BYEON[1], S. YADAV[2];
[1]Univ. of Washington Grad. Program In Neurosci., Seattle, WA; [2]Pharmacol., Univ. of Washington, Seattle, WA

Abstract: The autism risk gene *TAOK2* encodes a serine/threonine kinase important for several aspects of neurodevelopment including axon elongation, dendritic branching, and spine formation. We have recently discovered that TAOK2 is an endoplasmic reticulum (ER) membrane localized protein kinase that tethers the ER membranes to microtubules. Despite its importance in neuronal development and the strong association of TAOK2 mutations with neurodevelopmental disorders, the molecular function of TAOK2 in neuronal development is not well understood. To explore how TAOK2 contributes to neurodevelopment, we generated a human TAOK2 knockout induced pluripotent stem (iPS) cell line, which was differentiated into neural progenitor cells and excitatory forebrain neurons. We found that TAOK2 KO iPS derived neural progenitor cells exhibit defects in ER-microtubule tethering compared to control. Further, we characterized the impact of autism associated *TAOK2* mutations on neurodevelopment. Using in vitro kinase assays, we found that *TAOK2* mutations differentially affect its catalytic activity and ER localization. Using time lapse confocal microscopy we found that disease mutants perturbed ER dynamics within the neurites and dendritic spines of neurons. Our results suggest that through regulation of ER-MT tethering, TAOK2 mediates dendritic growth and spine formation in excitatory neurons.

Disclosures: S. Byeon: None. **S. Yadav:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.05

Topic: A.07. Developmental Disorders

Support: DA044308
DA051551

Title: Gut Microbial Metabolite Acetate Reverses Social Deficits in a Mouse Model of Autism Spectrum Disorder

Authors: *A. OSMAN[1], B. POLLACK[2], N. MERVOSH[2], K. MECKEL[2], M. BREEN[2], C. THAISS[3], L. LITICHEVSKIY[3], J. BUXBAUM[2], E. DRAPEAU[2], D. KIRALY[2];
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Abstract: Evidence demonstrates a role for the gut microbiome in Autism Spectrum Disorder (ASD), with signaling via microbial metabolites such as Short chain fatty acids (SCFAs) including acetate as a potential mechanism. In order to investigate microbiome and metabolite effects in a model for ASD we utilized a gene x microbiome paradigm in which we combined antibiotic (Abx) depletion of the microbiome with acetate replenishment in the Shank3 deletion (Shank3KO) model for ASD. In addition, to assess the clinical relevance of findings from the mouse model, targeted serum SCFA metabolomic analysis in Phelan McDermid (PMS) patients, who are hemizygous for the Shank3 gene, was conducted. Shank3KO mice and wild-type (Wt) littermates were divided into control, Abx depletion, acetate replenishment and acetate + Abx groups at weaning. On postnatal day 60, animals were subjected to behavioral testing using three-chambered social interaction. Caecal content was collected for 16S sequencing, metagenomic analysis and metabolomic profiling. Medial prefrontal cortex (mPFC) taken for gene expression profiling and western blot analysis. Serum from male and female PMS patients and their control counterparts was collected for targeted SCFA analysis. Analysis of mouse microbiome contents revealed a Shank3KO gene effect leading to decreased microbiome diversity, altered Bacteroidetes to Firmicutes ratios, and reduced levels of Lactobacillus genus bacteria. Metagenomic profiling revealed altered microbial functional output including fatty acid metabolism, this was confirmed by metabolomic analysis as reduced levels of SCFAs including acetate, effects that were exacerbated by Abx treatment. Behaviorally, control KO mice demonstrated decreased social interaction, a deficit exacerbated by microbiome depletion. RNA-sequencing showed marked changes in gene expression related to epigenetic regulation and synaptic plasticity in Abx+KO mice compared to Wt. Treatment of KO animals with acetate or Abx + acetate reversed the social deficits reported accompanied by changes to acetylation of specific histone marks. Human PMS patients were found to have sex-specific alterations in SCFA levels including acetate, which correlated with clinical behavioral measures. Our rodent data identified an altered gut microbiome and acetate levels in the Shank3KO model which can be targeted to reverse core social deficits possibly via epigenetic mechanisms. Clinical data corroborate findings of altered gut derived metabolites in PMS and add sex differences as a variable for further investigation.

Disclosures: A. Osman: None. **B. Pollack:** None. **N. Mervosh:** None. **K. Meckel:** None. **M. Breen:** None. **C. Thaiss:** None. **L. Litichevskiy:** None. **J. Buxbaum:** None. **E. Drapeau:** None. **D. Kiraly:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.06

Topic: A.07. Developmental Disorders

Support: NIH R01MH116002
Binational Science Foundation
Kavli Foundation
National Genetics Foundation
Simons Foundation
Spector Fund
Swebilius Foundation

Title: High-throughput functional analysis of autism risk genes

Authors: H. WEINSCHUTZ MENDES[1], W. WU[1], T. CHEN[1], D. JIN[1], S. FITZPATRICK[1], U. NEELAKANTAN[1], N. LI[1], M. CARLSON[1], A. PRUITT[1], I. NAWABUDIKE[1], K. ENRIQUEZ[1], J. RIHEL[2], X. PAPADEMETRIS[1], Z. WANG[1], ***E. HOFFMAN**[1];
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Abstract: Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders associated with deficits in social behavior, communication, and repetitive, restrictive behaviors. Despite advances in our ability to identify a growing number of ASD-associated risk genes, we lack pharmacological interventions targeting the core deficits in ASD, due in large part to our limited understanding of the underlying neurodevelopment mechanisms. In this study, we capitalize on the transparency and tractability of zebrafish as a vertebrate system to investigate the effects of loss of function of 10 high confidence ASD genes in parallel. Using CRISPR/Cas9 technology, we generated zebrafish mutants carrying frameshift mutations in the orthologs of 10 ASD risk genes. First, to investigate the effects of gene disruption on simple behaviors, we performed high-throughput behavior-based assays analyzing sensory processing and rest-wake behaviors. We identified unique and overlapping phenotypes across ASD risk gene mutants. Second, to identify brain activity phenotypes, we performed whole-brain activity mapping using immunohistochemistry to label active (pERK) and all (tERK) neurons. We found that zebrafish mutants display alterations in baseline brain activity. Third, to identify potential pharmacological suppressors, we screened over 750 FDA-approved compounds to identify their effects on behavior in wild-type fish. By comparing the fingerprints of these drugs in wild-type fish to the behavioral profiles of ASD risk gene mutants, we aim to identify compounds that rescue mutant behavioral phenotypes. Together, this research leverages the zebrafish system to identify mechanisms by which ASD risk gene disruption alters basic neurodevelopmental circuits and aims to identify potential drug candidates for further investigation.

Disclosures: H. Weinschutz Mendes: None. **W. Wu:** None. **T. Chen:** None. **D. Jin:** None. **S. Fitzpatrick:** None. **U. Neelakantan:** None. **N. Li:** None. **M. Carlson:** None. **A. Pruitt:** None. **I. Nawabudike:** None. **K. Enriquez:** None. **J. Rihel:** None. **X. Papademetris:** None. **Z. Wang:** None. **E. Hoffman:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.07

Topic: A.07. Developmental Disorders

Support: Canada Research Chair in Neurodevelopmental Disorders, CFREF (Institute For Data Valorization - IVADO), Brain Canada Multi-Investigator Research Initiative (MIRI).
Simons Foundation Grant Nos. SFARI219193 and SFARI274424
Funded in part by NIH grants U54 EB020403 and P41 EB015922
Swiss National Science Foundation (SNSF) Marie Heim Vögtlin Grant (PMPDP3_171331)

Title: Advanced Diffusion MRI Modeling Detects Altered Axonal Density in Carriers of 16p11.2 Copy Number Variants (CNV).

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[1]Imaging Genet. Center, Mark and Mary Stevens Neuroimaging and Informatics Inst., USC, Marina del Rey, CA; [2]Imaging and Genet. Center, Stevens Neuroimaging & Informatics Inst., [3]USC, Marina Del Rey, CA; [4]Pasteur Inst., Paris, France; [5]Ctr. Cantonale Autisme, Lausanne Univ. Hosp., Lausanne, Switzerland; [6]Univ. of Montreal, Montreal, QC, Canada; [7]Lausanne Univ. Hosp., Lausanne, Switzerland; [8]Univ. of Lausanne, Lausanne, Switzerland; [9]UCLA, Los Angeles, CA; [10]Stevens Inst. for Neuroimaging & Informatics, Univ. of Southern California (USC), Marina Del Rey, CA

Abstract: Introduction: Deletions and duplications of the chromosome 16p11.2 locus are associated with autism spectrum disorders, schizophrenia, cognitive and language deficits. Diffusion tensor imaging (DTI) studies have reported increases and decreases in fractional anisotropy (FA) in 16p11.2 deletion (16pDel) and 16p11.2 duplication (16pDup) respectively, with respect to healthy controls (HC) in deep white matter (WM) [1]. Here we analyzed data from five independent cohorts worldwide and reported WM alterations in the largest diffusion MRI (dMRI) study of 16p11.2 CNV carriers to date. We used advanced dMRI models that are highly sensitive to axonal density (AD), to avoid established biases of the DTI model. Methods: We measured axonal density with Diffusion Kurtosis (DKI), Mean Apparent Propagator (MAP) and Neurite Orientation Dispersion and Density Imaging (NODDI). We analyzed 19 WM regions of interest using the ENIGMA-DTI protocol [2]. Differences between 16pDel or 16pDup and HC were investigated using multiple linear regression with an HC4 method to account for heteroscedasticity [3]. dMRI measures were harmonized across sites using ComBat [4]. We used the following model and calculated the effect sizes of group differences: DTI-ROI-measure =

ß$_1$Diagnosis + ß^{2}Sex + ß$_3$Age + ß$_4$Age^2 + ß$_5$Mean-ROIs. Demographics: 70 individuals with a 16pDel (mean age: 14.0±7.3, 45M/25F); 63 individuals with a 16pDup (mean age: age 27.9±13.9, 40M/23F); 233 healthy controls (mean age: 28.8.0±15.5, 119M/114F).
Results: Higher AD was found in 16pDel in the cingulum, tapetum, and corona radiata and corpus callosum body, and lower AD in the superior longitudinal fasciculus. In 16pDup, lower AD was found in the cingulum, uncinate, and external capsule, and higher AD in the superior fronto-occipital fasciculus (Figure 1). Conclusions: AD is affected in both 16p11.2 CNV syndromes. Differences in AD between 16p11.2 CNV and HC were similar across dMRI measures, including mirror effects in the hippocampal cingulum (CGH).
[1] Chang et al. 2016 [2] Jahanshad et al. 2013 [3] Godfrey, 2006 [4] Fortin et al. 2017

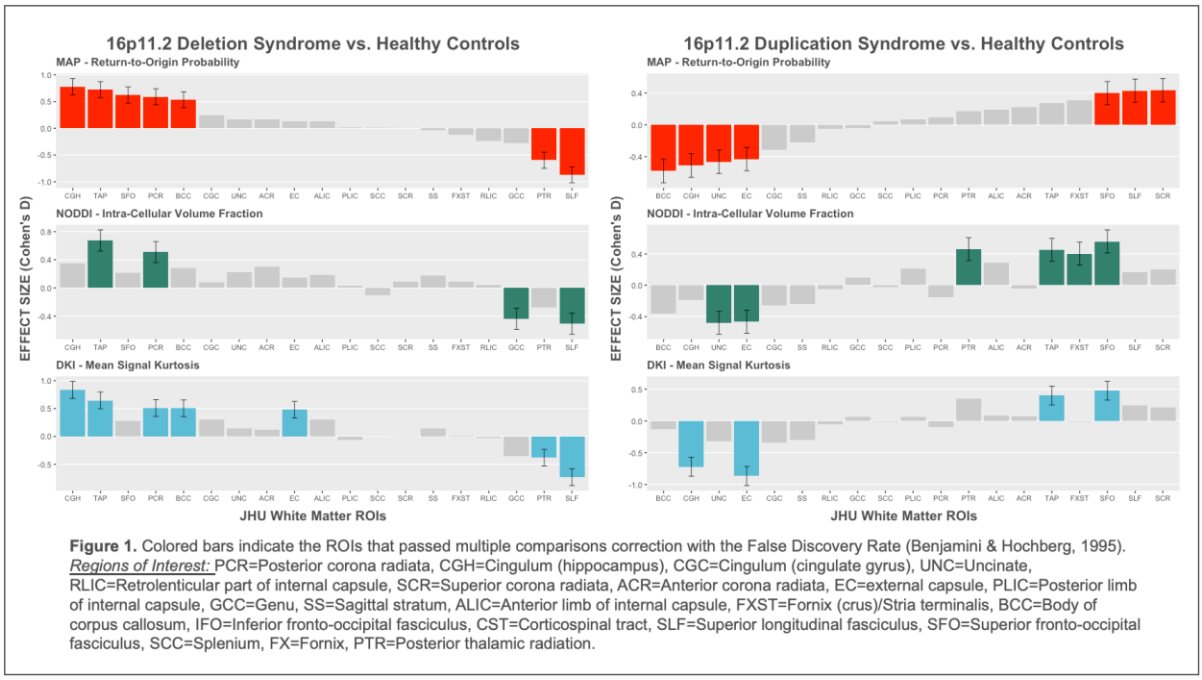


Figure 1. Colored bars indicate the ROIs that passed multiple comparisons correction with the False Discovery Rate (Benjamini & Hochberg, 1995). *Regions of Interest:* PCR=Posterior corona radiata, CGH=Cingulum (hippocampus), CGC=Cingulum (cingulate gyrus), UNC=Uncinate, RLIC=Retrolenticular part of internal capsule, SCR=Superior corona radiata, ACR=Anterior corona radiata, EC=external capsule, PLIC=Posterior limb of internal capsule, GCC=Genu, SS=Sagittal stratum, ALIC=Anterior limb of internal capsule, FXST=Fornix (crus)/Stria terminalis, BCC=Body of corpus callosum, IFO=Inferior fronto-occipital fasciculus, CST=Corticospinal tract, SLF=Superior longitudinal fasciculus, SFO=Superior fronto-occipital fasciculus, SCC=Splenium, FX=Fornix, PTR=Posterior thalamic radiation.

Disclosures: J.E. Villalon-Reina: None. **C. Moreau:** None. **T.M. Nir:** None. **D. Romascano:** None. **S. Lippe:** None. **N. Jahanshad:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen. **A. Maillard:** None. **B. Draganski:** None. **C.E. Bearden:** None. **S. Jacquemont:** None. **P.M. Thompson:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.08

Topic: A.07. Developmental Disorders

Support: Dup15q Alliance Predoctoral Fellowship 20182241
Neurobehavioral Genetics Predoctoral Training Grant 5T32MH073526
Intellectual Development and Disabilities Research Center (IDDRC) 5U54HD087101-03

Title: Electrophysiological Biomarkers of Sleep and Cognition in Children with Neurodevelopmental Disorders

Authors: *V. SARAVANAPANDIAN[1], D. NADKARNI[2], S. S. JESTE[3];
[1]Psychiatry, UCLA, Los Angeles, CA; [2]Pediatric Neurol., Children's Hosp. Med. Ctr. of Akron, Akron, OH; [3]A7-469, UCLA Semel Inst. of Biobehavioral Sci., Los Angeles, CA

Abstract: Despite the high prevalence of sleep disturbances and cognitive deficits in children with neurodevelopmental disorders (NDDs), very few studies have examined the correlation between altered sleep physiology and cognition in NDDs. This study explores the relationship between abnormal sleep physiology and neurodevelopmental phenotype in children with 15q 11.2-13.1 duplications (Dup15q syndrome), a genetic disorder highly penetrant for intellectual disability, autism, hypotonia, and epilepsy. Genes in the 15q region, particularly UBE3A and a cluster of $GABA_A$ receptor genes, are critical for neural development, disrupting synaptic protein synthesis and degradation as well as inhibitory neurotransmission. Children with Dup15q syndrome have abnormal sleep physiology including reduced sleep spindles, reduced slow wave sleep (SWS), and increased beta band oscillations (12-30 Hz). Considering the role of GABAergic neurotransmission in the generation of beta oscillations and in healthy NREM sleep physiology, these EEG signatures we see in Dup15q syndrome, together, likely reflect aberrant GABAergic neurotransmission. We analyzed 7 hours of overnight sleep EEG recording from a cohort of children with Dup15q syndrome (n=23, mean age: 5.9 years). We computed beta power in sleep, spindle density and %SWS, and investigated the relationship between these sleep metrics to quantitative measures of cognition, adaptive skills and sleep behavior. While we found no significant correlations with spindle density, we found that %SWS correlated with verbal cognition (R^2=0.32, p=0.04) and daytime sleepiness (R^2=0.36, p=0.04), and beta power correlated with daytime sleepiness (R^2=0.43, p=0.02). A better understanding of the relationship between impaired sleep and cognition in this condition will provide further understanding into the pathophysiology associated with the syndrome and offer insights into potential treatments that target sleep, and ultimately, improve cognitive and developmental outcomes in children with Dup15q syndrome.

Disclosures: V. Saravanapandian: None. **D. Nadkarni:** None. **S.S. Jeste:** None.

Poster

P043. Autism: Genetic Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P043.09

Topic: A.07. Developmental Disorders

Support: Washington University (WU) Children's Discovery Institute (CDI) and Maximizing Access to Research Careers (MARC)-Undergraduate Student Training in Academic Research (U-STAR) program
National Institutes of Health [U01 HG007530, U54 HD087011-S1, and R56/R01NS114551]
M-CM Network, the Jakob Gene Fund
Centene Corporation-WU Collaboration
WU Center for Regenerative Medicine
WU Institute for Clinical and Translational Sciences

Title: High-throughput identification of de novo regulatory mutations contributing to autism and intellectual disability

Authors: *I. ANTONY, E. M. A. LEWIS, T. TURNER, K. KROLL;
Washington Univ. in St. Louis, St. Louis, MO

Abstract: Autism spectrum disorder (ASD) impacts 1:54 American children and 1:160 children worldwide. Due to its high societal and individual costs, autism research must be prioritized in the global health sphere. A largely unexplored area is how mutations in regions of the genome that do not encode proteins play a role in disease pathology. Although genome-wide association studies have identified hundreds of single nucleotide variants (SNVs) associated with ASD, it is difficult to identify which SNVs functionally contribute to disease. We hypothesize that many noncoding ASD risk variants are in enhancer elements and alter expression of genes under their control by disrupting transcription factor binding. To test this model, we analyzed *de novo* SNVs in 2,671 individuals with autism and their unaffected siblings. We prioritized putative causal mutations by integrating SNVs identified in individuals with autism with functional genomic data from fetal brain tissue and induced pluripotent stem cell (iPSC)-derived neurons. These datasets included chromatin enrichment for the histone modification H3K27ac, which marks active enhancers, and accessible chromatin, as defined by ATAC-seq. When compared to sibling controls, autistic probands are over-represented for both SNVs found in regions with H3K27 acetylation and for SNVs found in known enhancers. Critically, the greatest over-representation of SNVs in ASD probands occurs in syndromic ASD genes identified by the SFARI Gene database, hinting that many of these SNVs contribute directly to ASD. Our preliminary data thus supports a model in which functional *de novo* noncoding variants perturb enhancer activity and thereby alter transcriptional regulation of genes with statistical enrichment for protein-coding de novo variants. To test this model, we are designing a Massively Parallel Reporter Assay (MPRA)

to define quantitative effects of these putative ASD risk alleles on enhancer activity and downstream gene expression. This high-throughput approach will identify noncoding SNVs that lead to autism, advancing our understanding of how altered gene regulation disrupts neurodevelopmental gene regulatory networks in ASD. We also expect our experimental approach to be applicable to identifying and characterizing noncoding mutations that contribute to other human diseases and disorders.

Disclosures: I. Antony: None. **E.M.A. Lewis:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **T. Turner:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **K. Kroll:** A. Employment/Salary (full or part-time):; Washington University in St. Louis.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: The association of HIV, mood disorders, and neurotransmitter metabolism in HIV-infected individuals under effective ART.

Authors: *R. LUU[1], S. SPUDICH[2], E. EUGENIN[1];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]Yale Sch. of Med., New Haven, CT

Abstract: The association of HIV, mood disorders, and neurotransmitter metabolism in HIV-infected individuals under effective ART. Ross.H. Luu[1], Serena Spudich[2], and Eliseo.A. Eugenin[1] [1]University of Texas Medical Branch (UTMB), Department of Neuroscience, Cell Biology and Anatomy, University of Texas Medical Branch, Galveston, TX, USA.[2]Yale School of Medicine, Department of Neurology, Yale University, New Haven, CT, USA.**Disclosures**R.H. Luu: None. E.A. Eugenin: None.**Abstract**Cocaine dysregulates brain activity and promotes extensive neurotransmitter activity changes resulting in compromised judgment and higher risks of contracting sexually transmitted diseases, including HIV. In this work, we examined the short term effects of cocaine abuse in neurotransmitters in HIV-infected individuals, including neurotransmitter metabolism and associated transcription regulation. Here, we determine that cocaine had acute transcriptional effects on HIV replication and long-term HIV replication has profound alterations in neurotransmitter metabolism even in the absence of HIV replication and transcription of key nuclear genes. We used HPLC to determine the presence and metabolism of dopamine, serotonin, and noradrenergic pathways in the serum of HIV-infected individuals under ART for extended periods. Mood disorders further compromised the metabolism of these catecholamine pathways suggesting profound effects on synaptic dysregulation even in viral replication. We determine that cocaine, a drug that further

compromises neurotransmitter metabolism, acutely increased viral replication, suggesting an intersection between HIV, long-term infection, ART, depression, and viral reactivation.
Funding: We would like to thank National NeuroAIDS Tissue Consortium (NNTC) and CHARTER for providing all human samples. This work was funded by The National Institute of Mental Health grant, MH096625, The National Institute of Neurological Disorders and Stroke, NS105584, and UTMB funding (to E.A.E).

Disclosures: R. Luu: None. **S. Spudich:** None. **E. Eugenin:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH-NIMHD-RCMIGrant No. 5G12MD007592
NIH Grant 226141323A

Title: Glycine Transporter 1 expression in the basal ganglia

Authors: *R. PEREZ[1], M. MIRANDA[2];
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Abstract: Glycine is an inhibitory neurotransmitter that participates in the regulation of many sensory and motor functions. It is cleared from the synaptic cleft by two different plasma membrane transporters, glycine transporter 1 (GlyT1) and glycine transporter 2 (GlyT2). These transporters share similarities in the localization within the central nervous system (CNS) but the organization and localization of the cells expressing them still require further investigation. GlyT2 is known as a neuronal marker and functions to recycle glycine into presynaptic neurons. On the other hand, GlyT1 is recognized as a glial marker and is important for fast transport of glycine from the synaptic cleft and termination of neurotransmission. A few studies have focused on the study of GlyT1 due to the historical concept of being expressed only in glial cells. Therefore, the objective of our study will be to identify the cells expressing GlyT1 in the mouse forebrain and midbrain using traditional IHC and transgenic technology. To date, our results in tissue sections suggest the presence of cell bodies labeled with Neuronal Nuclei antibody (Ab) in the subthalamic nuclei (STN) and substantia nigra (SN) and plasma membrane GlyT1. Staining with glial GFAP has shown poor colocalization of GlyT1 in astrocytes. Furthermore, to confirm these data, a knock-in mouse was generated to label GlyT1-positive cells with the reporter td-Tomato. Preliminary results have shown tdTomato expression in neuronal cell bodies labeled also with NeuN and GlyT1 antibodies in the STN and SN. Additional characterization of these neurons will be further analyzed by intracranial delivery of adeno-associated viral particles

(AAV's) and expression of the reporter in glial or neuronal cells. Altogether, these data suggest that GlyT1 can be expressed in neurons but their localization and function are yet unknown.

Disclosures: R. Perez: None. **M. Miranda:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant R01MH110681

Title: 5ht-type-2a receptor abnormalities in a mouse model of nmda receptor hypofunction relevant to schizophrenia

Authors: M. SINGH[1], K. NAKAO[1], J. J. HABLITZ[3], K. NAKAZAWA[2];
[1]Neurosci., [2]Southern Res., Birmingham, AL; [3]Dept. of Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Schizophrenia (SCZ) is a severe psychiatric disorder characterized by positive, negative and cognitive symptoms. Theories regarding etiology of SCZ have focused on the role of cortical dysfunction. Accumulating evidence suggests N-methyl-D-aspartate receptor (NMDAR) hypofunction in cortical interneurons in SCZ. We generated a conditional transgenic mouse, in which the Grin1 gene is disrupted from PV interneurons. *In vivo* local field potential (LFP) recording from auditory cortex of Grin1 mutant showed robust N1-like spontaneous LFP spikes during inter-stimulus intervals (ISIs) in response to periodic 40-Hz click train stimuli, suggesting induced-cortical hyperexcitability. *In vivo* microdialysis revealed increase of 5HT in auditory cortex of Grin1 mutant mice after acoustic stimulation. Slice electrophysiology recording of cortical pyramidal neurons (PyN) from Grin1 mutant mice conditioned with acoustic stimulation showed increased action potential firing (AP frequency), suggesting neuronal hyper excitability, control mice had no effect. TCB2, specific agonist of 5-HT2a receptor, mimicked acoustic effect in Grin1 mutant mice, indicating a role of 5-HT2a receptor in PyN hyperexcitability. Gαq-protein inhibitor YM254890 and GIRK channel activator ML297 completely reversed PyN excitability induced by acoustic stimulation. These results suggest that 5-HT receptors contribute to PyN excitation by activating Gα-protein pathway, subsequently inhibiting GIRK channel that causes increased neuronal firing. Increase in basal AP frequency of PyN in the Grin1 mutant mice was observe. Bicuculline, a GABAA receptor inhibitor, significantly increased AP frequency in control PyN after acoustic stimulation, no effect was observed in Grin1 mutant mice, suggesting chronic disinhibition caused increased excitability. Previous studies have shown that 5-HT2a receptors are involved in GABA release. Our

investigation revealed that TCB2 induced increase of sIPSC was abolished in Ppp1r2-Grin1 KO mice. Application of TCB2 increased sIPSC in floxed controls but not in Grin1 mutant mice. BIM-46187, a Gαq G protein inhibitor completely abolished the effect of TCB2 in control mice, indicating that activation of 5-HT2a receptor exerts its effect on presynaptic GABA release by activating Gαq protein pathway that is defected in Grin1 mutant. Present study provides an insight into the cause of PyN hyperexcitability after Grin1 deletion from PV interneurons, that is largely contributed by chronic disinhibition and impaired 5-HT2a receptor function in Grin1 mutant mice.

Disclosures: M. Singh: None. **K. Nakao:** None. **J.J. Hablitz:** None. **K. Nakazawa:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: This work was supported by Intramural Research at the NIH, NIMH.

Title: Mdma stimulates rhoa activity and neurotransmitter transporter internalization through the trace amine-associated receptor, TAAR1, in serotonin neurons

Authors: *S. M. UNDERHILL, S. G. AMARA;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The use of 3,4-methylenedioxymethamphetamine (MDMA) has recently gained interest as a therapeutic tool for the treatment of post-traumatic stress disorder (PTSD). However, the mechanism of action of this drug has not yet been fully elucidated. Here we demonstrate that MDMA acts by targeting the intracellular trace amine associated receptor 1 (TAAR1), an intracellular G-protein coupled receptor (GPCR) found throughout the brain. In previous studies, we have shown that the closely related compound, amphetamine (AMPH), enters neurons through the dopamine and norepinephrine transporters (DAT and NET) and activates TAAR1 which increases the activity of RhoA, a small GTPase that increases internalization of the DAT, NET and the neuronal glutamate transporter, EAAT3, resulting in an increase in extracellular DA, NE and glutamate concentrations. In this study, we show that MDMA enters serotonin neurons through the 5-HT transporter (SERT) and activates similar signaling cascades. Serotonin (5-HT) neurons in culture were identified by immunocytochemistry and functional SERT activity was confirmed by citalopram sensitive ^{3}H-5-HT uptake. Pre-treatment of these cultures for thirty minutes with 1 μM MDMA resulted in a decrease of SERT transport activity. This loss of function was blocked by an inhibitor of RhoA, indicating a similar biochemical cascade of that which we observed for DAT and NET in

response to AMPH. We confirmed that MDMA-induced loss of SERT function was due to trafficking of the SERT out of the plasma membrane by total internal reflection fluorescence (TIRF) microscopy in HEK293 cells. EAAT3 in these cells was also internalized in response to MDMA. In HEK293 cells in which the TAAR1 gene was deleted by CRISPR-Cas9, MDMA treatment did not lead to internalization of either transporter. MDMA-induced SERT and EAAT3 trafficking was examined in acute brain slices from wildtype or TAAR1 knockout mice brains. Slices containing the Raphe nucleus were treated with either vehicle control or MDMA (1 μM) and subsequently treated with a cell-impermeable biotin reagent that would label all surface proteins. By western blot analysis, we observed internalization of SERT and EAAT3 in response to the MDMA treatment. However, neither transporter exhibited any change in cell-surface expression in response to MDMA in the TAAR1 knockout tissue. These data indicate that pharmacological agents can regulate TAAR1 signaling in serotonergic neurons that may affect both serotonin and glutamatergic tone, indicating potential mechanisms to target for the development of novel drugs to treat PTSD.

Disclosures: S.M. Underhill: None. **S.G. Amara:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.05

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant DA038598 (GT)

Title: G protein βγ subunits interact with the serotonin transporter to evoke serotonin efflux

Authors: N. AWADALLAH[1], J. PINO[2], L. GONZALEZ[1], ***G. TORRES**[1];
[1]CUNY Sch. of Med., New York, NY; [2]Univ. of Atacama, Copiapo, Chile

Abstract: Extracellular serotonin (5HT) levels in brain are primarily regulated via the reuptake of 5HT through the serotonin transporter (SERT). SERT is a dynamic 12 transmembrane domain protein that regulates that reuptake of 5HT from the extracellular space in a Na^+/Cl^- dependent manner. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, used in the treatment of psychiatric ailments, selectively bind to SERT and prevent the reuptake of 5HT. Highly potent and addictive drugs of abuse such as amphetamine and its analogues are known to switch the functional role of SERT and produce an efflux of 5HT rather than reuptake. However, the precise mechanism of this phenomenon is still unknown. Our group has shown that βγ subunits of G Proteins bind to the carboxy terminus of the dopamine transporter (DAT) and that activation βγ subunits results in DAT-mediated dopamine efflux, similarly to the action of amphetamine. Here, we show parallels between the dopamine and 5HT systems using CHO cells

and, *ex vivo* acute brain slices. First, Gβγ and SERT are co-immunoprecipitated together in both CHO cells and in hippocampal and prefrontal cortex rodent brain tissue. Pharmacological work shows that incubation in both AMPH and mSIRK, a known and Gβγ activator, significantly increase 5HT efflux and is further potentiated when the two are combined. Further, activation of Gβγ via mSIRK significantly increases 5HT efflux in hippocampal and prefrontal cortex acute brain slices as measured by high performance liquid chromatography (HPLC). Gallein, a Gβγ inhibitor, attenuates this response back to basal levels. Together, these results indicate that Gβγ binds SERT and that activation of Gβγ promotes 5HT efflux. Overall, further characterizing this mechanism may aid in establishing more specific pharmacotherapies for 5HT-related disorders.

Disclosures: N. Awadallah: None. **J. Pino:** None. **L. Gonzalez:** None. **G. Torres:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.06

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant R01NS112390
NIH Diversity Supplement 3R01NS112390-02S1
BHI Pilot Grant

Title: Novel optical reporter reveals compartmentalized trafficking of the dopamine transporter in neurons

Authors: *J. SAENZ, X. ZHOU, D. J. BARKER, E. DICICCO-BLOOM, P.-Y. PAN; Rutgers Univ., Piscataway, NJ

Abstract: The dopamine transporter (DAT) is a major gateway for DA uptake and termination of dopaminergic signaling in the brain. DAT trafficking is a highly regulated process and is implicated in a multitude of human conditions, such as cocaine addiction. While much of our understanding on constitutive and regulated DAT trafficking came from studies of heterologous cells, it remains unclear if central DA neurons, especially their dendrites and axons, share the same rules. We have now engineered a novel pH-sensitive reporter for DAT by conjugating pHluorin to the second exofacial loop of human DAT. We show that DAT-pHluorin is a superior tool to study DAT trafficking in neurons as it exhibits DA uptake, cocaine sensitivity as well as PI3K/PKC regulated DAT recycling similar to the endogenous protein. Unlike the homogenous expression of DAT in heterologous cells, midbrain neurons exhibit compartmentalized DAT expression. While dendrites and soma contain a higher fraction of recycling/degradative DAT in the acidic compartment, axons express most of the DAT on the plasma membrane. Large axonal varicosities may be active sites of DAT turnover, as a higher fraction of acidic DAT was found

in these structures compared to the axonal shaft. Furthermore, our data suggests that a 24-hour cocaine treatment induces a redistribution of the intracellular recycling DAT in the soma and dendrites to the surface of neurites. Thus, our study using the newly engineered DAT optical reporter reveals, for the first time, compartmentalized DAT trafficking rules in neurons.

Disclosures: J. Saenz: None. **X. Zhou:** None. **D.J. Barker:** None. **E. DiCicco-Bloom:** None. **P. Pan:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.07

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: BBSRC (BB/5015760/1)

Title: Gaba and glutamate are depolarising during early development of the hypothalamic arcuate nucleus in japanese quail (coturnix japonica).

Authors: *A. JOHNSTON, S. MEDDLE;
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Abstract: The regulation of feeding is a complex interaction between central and peripheral signalling governed centrally by the melanocortin system comprising specific neuronal populations and circuits in the hypothalamus and brainstem. Little is known about the central mechanisms regulating the development of appetite in birds in early life. Using whole cell patch clamp electrophysiology in Japanese quail brain slices containing the hypothalamic arcuate nucleus (ARC), we investigated the effects of GABA and glutamate from embryonic day 10 to post hatch day 5. The exogenous application of GABA caused a membrane depolarisation between embryonic day 14 and day 2 after hatch, when there is a depolarising to hyperpolarising shift in the response of ARC neurones to exogenous GABA. During the GABA-mediated depolarisation both spontaneous synaptic activity and action potential firing was reduced. By contrast, exogenous glutamate always depolarised ARC neurones and increased action potential firing at all developmental stages. The GABA-induced depolarisation was abolished by the specific $GABA_A$ receptor antagonist bicuculline. It was also absent in brain slices pre-incubated with verapamil and bumetanide, suggesting there is an influx of Ca^{2+} ions through L-type voltage-gated calcium channels during the $GABA_A$ receptor-mediated depolarisation. This depolarising action of exogenous GABA is correlated with the expression of the developmentally regulated NKCC1 Cl^- ion importer during avian brain development. We conclude that GABA is functionally inhibitory in the avian hypothalamic feeding circuits, but the inhibition is associated with a depolarisation during embryonic development. Glutamate is excitatory at all stages of

development to become the dominant excitatory neurotransmitter. Our data is in agreement with studies that identify GABA as predominantly depolarizing at the cellular level over a limited period of time and, at the same time, inhibitory at the network level during mammalian brain development. All work was performed under UK Home Office licence, ARRIVE guidelines and local ethical review. Research supported by BBSRC (BB/5015760/1).

Disclosures: A. Johnston: None. **S. Meddle:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.08

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Marie Skłodowska- Curie Actions, Grant/Award Number: H2020- MSCA- ITN- 2016- 722053
MSCT Research Excellence Programme 2020. REP-2020-006

Title: Gat1 uptake dysfunction in absence seizures

Authors: *T. P. MORAIS[1,2], C. C. PINA[3,4], M. N. SOTTOMAYOR[3,4], A. M. SEBASTIÃO[3,4], G. D. GIOVANNI[1,2], S. H. VAZ[3,4], V. CRUNELLI[1];
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Abstract: Absence Seizures (ASs) are genetic generalized non-convulsive paroxysms due to alterations in GABA neurotransmission. Administration of drugs that boost GABA levels elicits ASs in normal individuals while exacerbates them in Absence Epilepsy (AE) patients. Extrasynaptic GABA-A receptors ($GABA_A$Rs) tonic inhibition is increased in the thalamus of AE animal models, indicating that an increase, rather than a decrease, of $GABA_A$R inhibition, contributes to the pathophysiology of ASs. Extrasynaptic $GABA_A$Rs are activated by ambient GABA and the level of GABA is controlled by GABA transporters (GATs). An increase in tonic $GABA_A$R inhibition is necessary and sufficient for the appearance of experimental ASs. In different genetic animal models of AE, the increased tonic $GABA_A$R inhibition results from a malfunction in the GABA transporters type 1 (GAT1). Strikingly, no genetic abnormalities of the GAT1 gene have been identified in absence epilepsy animal models. In consideration of this, here we have investigated the nature of GAT1 malfunction in the polygenic rat model GAERS (Genetic Absence Epilepsy Rat from Strasbourg) and the monogenic mice model Stargazer (*stg*) to evaluate differences in GABA uptake in AE animals and to test whether the thalamic GAT1

malfunction resulted from a failure of trafficking to the outer astrocytic membrane or a misplaced localization concerning the synaptic or extrasynaptic space. We also assessed GAT3 function/localization for comparison.Our data show a decrease in total GABA uptake due to a smaller GAT1 mediated uptake in the motor cortex, somatosensory cortex, visual cortex, thalamus, and hippocampus of GAERS and stg, compared to their respective controls. The observed decrease in GAT1 uptake is not due to a trafficking failure and is not observed in the early stages of life before ictogenesis.

Disclosures: T.P. Morais: None. **C.C. Pina:** None. **M.N. Sottomayor:** None. **A.M. Sebastião:** None. **G.D. Giovanni:** None. **S.H. Vaz:** None. **V. Crunelli:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.09

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant R01-NS113600
NIH Grant R01-MH125772
Fondation pour la Recherche Médicale (Postdoctoral Fellowship for a research abroad)
Fondation Bettencourt Schueller (Prix pour les Jeunes Chercheurs 2016)
American Epilepsy Society Postdoctoral Research Fellowship (2020)

Title: Adenosine/a2a receptor retrograde signaling mediates presynaptic hippocampal ltp and facilitates epileptic seizures

Authors: *K. NASRALLAH[1], C. BERTHOUX[1], R. LUJAN[2], M. GULFO[1], P. E. CASTILLO[3]; [1]Albert Einstein Col. of Med., Bronx, NY; [2]Univ. de Castilla-La Mancha, Albacete, Spain; [3]Albert Einstein Coll Med., Bronx, NY

Abstract: The dentate gyrus (DG) of the hippocampus contains two types of excitatory neurons, granule cells (GCs) and mossy cells (MCs), which are reciprocally connected, thereby forming an associative circuit that has been implicated in DG-dependent memory and temporal lobe epilepsy. MCs can powerfully influence GC activity. Remarkably, a single MC contacts about 30,000 GCs. MC-GC synapses express a robust form of presynaptic long-term potentiation (MC-GC LTP) that increases DG output and may contribute to memory formation and epileptogenesis. We recently found that initial epileptic seizures trigger broad MC-GC LTP *in vivo*, which promotes further seizures. It is therefore crucial to understand the molecular processes underlying MC-GC synaptic strengthening. MC-GC LTP requires both *post*synaptic TrkB activation and *pre*synaptic cAMP/PKA signaling, indicating the involvement of a

retrograde messenger, whose identity remains unknown. Here, we addressed this knowledge gap using a combination of complementary approaches. Immunoelectron microscopy revealed the presence of Gs-coupled adenosine A_{2A} receptors (A_{2A}Rs) at MC axon boutons. Using selective pharmacology and a cell-specific *Adora2a* knockout strategy, we found that activation of A_{2A}Rs was necessary and sufficient to induce MC-GC LTP in acute rodent hippocampal slices. By expressing a genetically encoded adenosine sensor in MC axons, we found that LTP induction was associated with a transient, TrkB-dependent release of adenosine. Interfering with adenosine release from GCs abolished LTP. Lastly, adenosine release was also detected during acute epileptic seizures induced by intraperitoneal injection of kainic acid. Thus, adenosine/A_{2A}Rs emerges as a novel retrograde signaling mechanism that mediates presynaptic LTP in the hippocampus and that could be targeted for early intervention in epilepsy.

Disclosures: K. Nasrallah: None. **C. Berthoux:** None. **R. Lujan:** None. **M. Gulfo:** None. **P.E. Castillo:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.10

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: R01 DA035224
R21 DA049057

Title: Nociceptin-stimulated dopamine transporter trafficking and its impact on reward

Authors: *N. BOLDEN, E. KAHUNO, H. E. MELIKIAN;
Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Dopamine (DA) transmission is required for reward, motivation, and habit formation. The nucleus accumbens (NAcc) receives dense DAergic innervation via the mesolimbic circuit, and DA transmission in the NAcc is required for rewarding behaviors. DA signaling is restricted by the presynaptic DA transporter (DAT) which recaptures extracellular DA following synaptic release. DAT is potently inhibited by abused psychostimulants, such as cocaine, which binds to DAT and thereby increase extracellular DA. DAT surface expression is not static. DAT constitutively traffics via the endocytic recycling pathway, and both G_q- and $G_{i/o}$-coupled receptors modulate DAT surface expression by accelerating DAT internalization and surface delivery rates, respectively. Numerous studies have identified the mechanisms required for regulated DAT trafficking. Nevertheless, it remains unknown whether, or how, regulated DAT trafficking impacts DA signaling and DA-dependent behaviors, such as psychostimulant-mediated reward. In the current studies, we found that nociceptin receptor (NOPR) activation

rapidly delivers DAT to the plasma membrane in mouse *ex vivo* NAcc slices. NOPR activation in NAcc blocks cocaine's ability to increase extracellular DA, however the mechanism(s) responsible for this effect are poorly understood. We hypothesize that nociceptin-stimulated DAT surface delivery plays a central role in the effects of nociception in NAcc. Our ongoing studies leverage chemogenetics, conditional gene silencing, and *in vivo* conditional expression of trafficking dysregulated DAT mutants, to direct test whether: 1) nociceptin-stimulated DAT trafficking in NAcc is mediated by presynaptic NOPRs in DAergic terminals, 2) nociception infusion into NAcc suppresses cocaine-mediated reward, and 3) NOPR-stimulated DAT surface delivery is required for nociceptin to dampen reward. The results are expected to shed light on the role of regulated DAT trafficking in addictive behaviors.

Disclosures: N. Bolden: None. **E. Kahuno:** None. **H.E. Melikian:** None.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.11

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NeuroTrans, Grant agreement ID: 860954

Title: Solid supported membrane-based electrophysiology (SSME) electrophysiology meets SGLT1 and GAT1

Authors: A. BAZZONE[1], **R. ZERLOTTI**[1,2], *E. DRAGICEVIC[1], M. BARTHMES[1], N. FERTIG[1];
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Abstract: Transporter assays are often limited by the availability of labeled substrates and lack real-time data. Here, we developed functional assays to characterize the human $Na^+/Cl^-/\gamma$-aminobutyric acid (GABA) and Na^+/glucose co-transporters - GAT1 and SGLT1 - using solid supported membrane-based electrophysiology (SSME). This approach overcomes several bottlenecks of other techniques and provides new insights into GAT1 and SGLT1 transport mechanisms. In conventional electrophysiology voltage steps are used to trigger pre steady-state (PSS) and transport currents, which are commonly recorded in whole cells at a defined holding potential. Transport and PSS electrogenicity in SGLT1 and GAT1 triggered by voltage steps is postulated to be a result of transitions within the sugar and GABA-free carriers, e.g. the alternating access of the charged sodium binding sites within the empty carrier. In contrast, SSME utilizes membrane vesicles at 0 mV and the transport cycle is triggered by applying a substrate concentration gradient as the main driving force. Using SSME, we observed substrate-

induced PSS currents, most likely representing conformational transitions within the substrate-loaded carrier, which are not observed with conventional electrophysiology. We examined the impact of different driving forces on influx, efflux, and PSS currents, focusing on sodium gradients and membrane voltage. We found that internal accumulation of sodium strongly reduces Vmax, rendering sodium release rate limiting at 0 mV. Application of membrane voltage only affected the apparent KM in SGLT1, but Vmax in GAT1. We also found that transport properties in GAT1 mediated influx and efflux modes are highly asymmetric, while SGLT1 has similar properties for influx and efflux.

Disclosures: **A. Bazzone:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **R. Zerlotti:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. Barthmes:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

P084. Amino Acids and Other Neurotransmitters

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P084.12

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: The National Centre for Research (NCBiR) Polish Norwegian Research Program: Pol-Nor/196190/23/2013
South-Eastern Norway Regional Health Authority: 21561/2017052
UiO:Life Science

Title: Ammonium ion (NH_4^+) impairs Slc38 glutamine transporter function contributing to development of hepatic encephalopathy

Authors: *F. A. CHAUDHRY[1], E. H. HAMDANI[2], M. POPEK[3], M. FRONTCZAK-BANIEWICZ[3], M. N. MOEN[1], T. P. UTHEIM[4], J. ALBRECHT[3], M. ZIELINSKA[3];
[1]Univ. of Oslo, Oslo, Norway; [2]Oslo Metropolitan Univ., Oslo, Norway; [3]Mossakowski Med. Res. Inst. PAS, Warwaw, Poland; [4]Oslo Univ. Hosp., Oslo, Norway

Abstract: In liver failure, ammonia escapes detoxification in the liver and accumulates in the cerebrospinal fluid (CSF) where it is considered to be the main pathogenic toxin in hepatic encephalopathy (HE), a neuropsychiatric disorder belonging to the group of diseases classified as hyperammonemic encephalopathies. However, the molecular mechanisms involved have been disputed. We have previously shown that four members of the solute carrier 38 (Slc38) family of amino acid/glutamine transporters contribute to astroglial-to-neuronal shuttling of glutamine for the synthesis of glutamate and GABA. As HE is associated with dysfunctional glutamatergic and

GABAergic neurotransmission, we investigated whether ammonia perturbs the Slc38 transporters and whether this contributes to HE patho-physiology. We now show by two-electrode voltage-clamp electrophysiology on *Xenopus laevis* oocytes expressing Slc38 transporters that ammonium ions compete at the glutamine binding sites of the system N transporters Slc38a3 and Slc38a5 and thereby inhibit bidirectional astroglial glutamine transport. Ammonium ions also permeate uncoupled through the same transporters in competition with H^+, Na^+ and K^+ and may perturb astroglial intracellular pH, membrane potential and K^+-buffering. To further test a relation between Slc38a3 and HE, we knocked-down Slc38a3 by transcranial injection of *vivo morpholino* (VM) oligomers in mice. This results in cerebral cortical edema, lower extracellular glutamine content and reduced total brain glutamate, indicative of disrupted neurotransmitter synthesis, all mimicking events contributing to HE development. Finally, in the azoxymethane (AOM) mouse model of acute liver failure (ALF) we demonstrate downregulation of Slc38a3 protein and impeded astroglial glutamine release followed by cytotoxic edema. Altogether, our data mechanistically demonstrate differential regulation of four Slc38 glutamine transporters by NH_4^+ and suggest a primary role of these transporters in the pathophysiology of HE.

Disclosures: F.A. Chaudhry: None. **E.H. Hamdani:** None. **M. Popek:** None. **M. Frontczak-Baniewicz:** None. **M.N. Moen:** None. **T.P. Utheim:** None. **J. Albrecht:** None. **M. Zielinska:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.01

Topic: B.10. Epilepsy

Support: NINDS grant #1U54NS079202

Title: Allopregnanolone terminates benzodiazepine-resistant tetramethylenedisulfotetramine (TETS)-induced status epilepticus in mice after failed midazolam treatment

Authors: *D. ZOLKOWSKA, R. JUNG, M. A. ROGAWSKI;
Dept. of Neurol., Sch. of Med., Univ. of California, Davis, Sacramento, CA

Abstract: Tetramethylenedisulfotetramine (TETS), a potent GABA-A receptor antagonist, is a highly lethal neurotoxin that induces convulsive status epilepticus (SE) in humans that is often refractory to treatment. Benzodiazepines such as midazolam (MDZ) are used for initial treatment of SE, including following a chemical exposure emergency such as with TETS. Often seizure termination fails to occur, especially when therapy is delayed. Current federal guidelines for the pre-hospital treatment of chemical exposure induced SE recommend treatment with a 10 mg

dose of MDZ administered intramuscularly (IM), followed 10 min later by a second 10 mg dose MDZ if the first one is not effective. Our previous studies have shown that allopregnanolone (5α,3α-P), a neurosteroid positive modulator of synaptic and extrasynaptic GABA-A receptors, may provide an effective treatment option in benzodiazepine refractory SE, including TETS-induced SE. In the present study, we determined whether IM 5α,3α-P (12 mg/kg) injected 10 min after IM MDZ (0.23 mg/kg) was more effective than 2 injections of IM midazolam in terminating TETS-induced SE in mice. To create a model mimicking the SE produced by TETS in humans, mice implanted with EEG electrodes were pretreated with a single dose of riluzole (10 mg/kg, IP) and 10 min later received a lethal dose of TETS (0.2 mg/kg, IP). Riluzole does not inhibit TETS-induced SE but does protect against the rapidly lethal effects of TETS in mice, providing a model of persistent seizure activity. Video EEG recording was carried out for at least 4 h after exposure to TETS. Animals were monitored for survival for 72 h after treatment. MDZ was administered at 40 min after the first myoclonic twitch and 10 min later another dose of MDZ (0.23 mg/kg IM) or 5α,3α-P (12 mg/kg, IM) was administered. In mice treated with 2 doses of MDZ, persistent convulsive seizures were not terminated. The animals did not appear to be visibly sedated at any time following the dual MDZ treatment. Addition of 5α,3α-P after MDZ, led to rapid termination of behavioral and electrographic SE as assessed by EEG power. The latter treatment terminated persistent seizures within 6.7 ± 2.4 min and increased 72 h survival from 0% (2 doses of MDZ) to 100%. Animals receiving MDZ and 5α,3α-P experienced sedation with loss of righting reflex for 1.19 ± 0.12 h, but fully recovered. Our results demonstrate that replacement of the second dose of MDZ with 5α,3α-P overcomes benzodiazepine resistant TETS-induced SE and effectively terminates TETS-induced behavioral and EEG seizures. Transient sedation occurred with adjunctive 5α,3α-P but all animals fully recovered without apparent long-term adverse effects.

Disclosures: D. Zolkowska: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents assigned to the Regents of Univ. of California. **R. Jung:** None. **M.A. Rogawski:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents assigned to the Regents of Univ. of California.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.02

Topic: B.10. Epilepsy

Support: R01 NS120945
UVA Brain Institute

Title: Status epilepticus treatment by metabolic modulators

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[1]Univ. of Virginia, Charlottesville, VA; [2]Dept Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA

Abstract: Rationale: We tested whether β-hydroxybutyrate (BHB) and the specific lactate dehydrogenase (LDH) inhibitor, oxamate, can terminate Status Epilepticus (SE), which is a neurological emergency requiring rapid seizure termination to protect life. The ketogenic diet is a promising remedy for patients with refractory SE. However, it takes time to attain ketosis in patients with ongoing SE. Therefore, drugs that could mimic the therapeutic effect of KD action but act much faster are desirable. **Methods:** SE was induced by continuous hippocampal stimulation as previously described (Lewczuk et. al. 2018). Briefly, we stereotaxically implanted 7-8 weeks old C57Bl/6 mice with a bipolar insulated stainless-steel electrode in the left hippocampus, bilateral supra-dural cortical electrodes, and a cerebellar reference electrode. A week after the electrode implantation, we connected the animals to a video-EEG monitoring system and set stimulation intensity to twice the after-discharge threshold (ADT). We applied 10 sec stimulus trains followed by a 5 sec off period for 60 min. We injected mice intraperitoneally with BHB (1g/kg), oxamate (1g/kg), or saline 15 minutes after the development of autonomous seizure. We evaluated the duration and severity of EEG and scoring behavior on the Racine scale. **Results:** Oxamate treatment significantly shortened SE duration in comparison to saline-injected control mice (median SE duration in saline- versus oxamate-treated mice: 263.5 and 63 minutes respectively; n = 5 each, p = 0.0233). Moreover, severe behavioral seizures (grades 4 and 5) were less frequent following oxamate injection. The oxamate-treated animals returned to normal behavior faster than control mice (n = 5 each, p = 0.0033). In addition to changes in behavioral manifestations, we detected changes in EEG with fewer high-frequency discharges and power EEG attenuation quickly following oxamate treatment. Similarly, SE duration in BHB-treated mice was significantly shorter than saline-injected mice (median SE duration in saline- versus BHB-treated mice: 240 and 100 minutes respectively p = 0.0289; n = 8 each). In addition, the median behavioral seizure scores were lower in the BHB-treated group when compared to saline-injected mice (n = 6 each, p=0.002). We observed a gradual attenuation of power EEG and high-frequency discharges along with seizure severity, which became less common in BHB-treated mice than control animals. **Conclusions:** Targeting metabolic pathways by directly affecting the metabolite concertation (BHB) or enzyme activity (LDH) represents a promising strategy to treat SE. Ongoing studies explore these drugs' effect on neuronal excitability.

Disclosures: D. Skwarzynska: None. **K. Rekawek:** None. **J. Kapur:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.03

Topic: B.10. Epilepsy

Title: Therapeutic monitoring using NMR based metabolomics in drug resistant epilepsy in a Ghanaian adult population

Authors: *P. K. MANTE, J. DANQUAH, M. ASARE;
Kwame Nkrumah Univ. of Sci. and Technol., Kumasi, Ghana

Abstract: It has been hypothesized that epilepsy patients present with unique chemical signatures and metabolic pathways in the brain and may influence efficacy of antiepileptic drugs (AEDs). The aim of our study is to assess the brain metabolic differences between the drug-responsive and drug-resistant Ghanaian epileptic patients in order to establish the metabolic background for drug-responsiveness in epilepsy. 30 drug-resistant epilepsy patients (median age: 23 years; males: 56%) and 31 drug-responsive patients (median age: 25 years; males: 49%) were clinically assessed with EEG. The control group consisted of 20 patients (median age: 21 years; males: 50%) with no neurological disorders. Serum samples were analyzed with ^{1}H-NMR on 400 MHz spectrometer and spectra analyzed using univariate and multivariate with the application of correction for age variation. Drug-resistant epilepsy patients group was exhibited by increased levels of serum creatine, glycine, lactate, lipids and glycoproteins; levels of citrate and choline decreased as compared to the drug-responsive and control groups. Levels of choline, glycine and lactate were significantly correlated with seizure control and number of AEDs in use by patients. NMR-based metabolomics could provide information on efficacy of antiepileptic drug treatment in epilepsy patients.

Disclosures: P.K. Mante: None. **J. Danquah:** None. **M. Asare:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.04

Topic: B.10. Epilepsy

Title: Gene profiles of levetiracetam treated responding vs. non-responding epileptic rats

Authors: *L. A. PICHARDO-MACIAS[1], S. JUÁREZ-MÉNDEZ[2], I. J. CONTRERAS-GARCÍA[4], V. VILLEGAS-RUÍZ[2], J. G. MENDOZA-TORREBLANCA[3], S. R. ZAMUDIO-HERNÁNDEZ[5];
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Autónoma Metropolitana Unidad Iztapalapa, CDMX, Mexico; [5]Fisiología, Inst. Politecnico Nacional, México, Mexico

Abstract: Levetiracetam (LEV) is an anticonvulsant drug effective for seizure control in patients and different animal models of epilepsy. However, approximately 30% of patients do not respond to LEV treatment. Until now, little is known about the resistance to LEV treatment. Thus, the objective of this study was to identify gene expression profiles in the dentate gyrus of epileptic rats that responded or did not respond to LEV treatment. For this purpose, male Wistar rats were divided into the following groups: Control + LEV (nonepileptic rats treated with the drug), R (LEV-responders) and NR (LEV non-responders). Epilepsy was induced by systemic administration of lithium plus pilocarpine and was determined by monitoring behavior using video recordings. LEV treatment (300 mg/kg/d) lasted one week and was accomplished with osmotic mini-pumps. After treatment, microarrays were used, together with a bioinformatic analysis based on Genetic Ontology categories, to identify alterations in the dentate gyrus genome among R and NR rats. The results showed more altered genes in the NR group (742) compared to the R group (418). Within the gene profile of NR rats, greater overexpression of genes related to inflammation, neuronal reorganization and astroglial activation was found (C1AQ, C1QB, C1QB, PTGS2, PTAFR, SOX11, BDNF, CD44, TESC, VIM, GSN, S100A10 and S100A4), while genes related to neurotransmission were more under-expressed (GABRD, GABRR3, GRM8, SYT4, SYT10). Only the R group overexpressed NPYR2, which may mediate a protective mechanism. Thus, effective LEV treatment increased NPY2R gene expression and decreased overexpression of genes related to the reorganization of neuronal circuits and the microenvironment, as well as to neuronal, glial and astrocytic hypertrophy.

Disclosures: L.A. Pichardo-Macias: None. **S. Juárez-Méndez:** None. **I.J. Contreras- García:** None. **V. Villegas-Ruíz:** None. **J.G. Mendoza-Torreblanca:** None. **S.R. Zamudio-Hernández:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.05

Topic: B.10. Epilepsy

Support: NIH NS50554
CMSRU/Rowan

Title: Modulation of opioidergic function alters electroshock seizure responses in mice

Authors: *T. N. FERRARO[1], J. R. DECHIARA[1], B. D. FISCHER[1], G. A. DOYLE[2], R. J. BUONO[1], W. H. BERRETTINI[2];

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Abstract: We studied the effect of opioidergic modulation on seizure responses to electroshock stimulation in DBA/2J (D2) and C57BL/6J (B6) mice using a combination of genetic and pharmacological approaches. In genetic studies, we show that constitutive deletion of the mu opioid receptor (MOR) gene, *Oprm1*, results in a significant reduction of maximal electroconvulsive shock (ECS) seizure threshold in homozygous *Oprm1* knockout mice compared to respective wild type litter mates independent of genetic background (i.e. D2 or B6). In pharmacological studies, we report that administration of morphine for 4 days via a subcutaneously implanted pellet (25 mg) significantly reduces the expression of maximal ECS seizures in both D2 and B6 wild type mice. Conversely, pretreatment of D2 and B6 mice with naltrexone (1-10 mg/kg, i.p.) increases maximal ECS seizure susceptibility in both strains. We also report that higher doses of naltrexone (100-300 mg/kg, i.p.) elicit generalized behavioral seizures, with D2 mice displaying significantly greater susceptibility than B6. Overall, our data show that inhibition of opioidergic neurotransmission mediated by the MOR, either genetically via gene knockout or pharmacologically via antagonist pretreatment, increases ECS seizure susceptibility in mice, whereas MOR activation via full agonist pretreatment decreases ECS seizure susceptibility. Further, the greater proconvulsant effect of high dose naltrexone in D2 compared to B6 mice suggests that endogenous opioids may be involved in the robust difference in seizure susceptibility between these two classic inbred mouse strains, or that non-specific or off-target drug action is involved. We conclude that modulation of opioidergic function represents a viable means of ameliorating seizures and treating epilepsy.

Disclosures: T.N. Ferraro: None. **J.R. DeChiara:** None. **B.D. Fischer:** None. **G.A. Doyle:** None. **R.J. Buono:** None. **W.H. Berrettini:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.06

Topic: B.10. Epilepsy

Support: Epilepsy Discovery Award, EP180036

Title: Activity enhancement by D cycloserine prevents post-stroke epilepsy.

Authors: *Y. ADHIKARI[1], X. JIN[2];
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Abstract: Homeostatic plasticity is a slow adaptive process that allows neurons to adjust their synaptic strength to compensate for perturbations in network activity. Homeostatic control of cortical excitability, connectivity and plasticity is crucial for normal brain functions. Abnormalities in homeostatic plasticity results in hyperexcitability of neuronal network which is a key pathological feature of epilepsy. After brain insults loss of neurons and synapses causes activity deprivation in the surviving neurons. To compensate for the lost function, the surviving neurons use the homeostatic plasticity mechanism to scale up excitation and/or scale down inhibition to attempt to reach the physiological range of neuronal activity. This causes transition of the surviving neurons from initial hypoactivity to hyperactivity state which increases the excitability of the network resulting in epileptogenesis. After stroke, a transient hyperactivation of NMDARs occurs which is followed by a profound and long-lasting loss of function of NMDARs. This decrease in functional NMDAR could result in activity deprivation in the surviving neurons, thereby triggering homeostatic plasticity . We hypothesize that enhancing NMDAR activity by D-cycloserine, (a partial NMDAR agonist) during this critical time window will compensate for the activity deprivation caused by loss of function of NMDARs . This would prevent homeostatic plasticity in surviving neurons and prevent post-stroke epilepsy. In this study we induced photochemical stroke in 8 weeks old YFP mice and administered (30mg/ kg/day, i.p.) from day 3-15 after stroke. We report evidence that DCS administration in this time window decreases seizure susceptibility after photochemical stroke. These findings identify activity enhancement as a target for therapeutic intervention to alleviate post stroke epilepsy.

Disclosures: Y. Adhikari: None. **X. Jin:** None.

Poster

P149. Antiepileptic Therapies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P149.07

Topic: B.10. Epilepsy

Support: MH119880

Title: Encircling supragranular laser scalpel cuts around a seizure focus attenuate seizure propagation without any behavioral effects in a mouse model of focal epilepsy

Authors: *S. LIEBERMAN[1], D. A. RIVERA[2], R. MORTON[2], A. HINGORANI[2], T. SOUTHARD[1], L. JOHNSON[3], J. REUKAUF[2], R. E. RADWANSKI[2], M. ZHAO[4], N. NISHIMURA[2], O. BRACKO[2], T. H. SCHWARTZ[5], C. B. SCHAFFER[2];
[1]Col. of Vet. Med., [2]Meinig Sch. of Biomed. Engin., [3]Statistical Consulting Unit, Cornell Univ., Ithaca, NY; [4]Dept Neurolog Surg, Weill Cornell Med. of Cornell Univ., New York, NY; [5]Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY

Abstract: About 5% of all epilepsy cases are due to traumatic brain injuries that lead to focal seizures. Focal epilepsy is characterized by seizures that initiate in one location and propagate out to larger areas. 45% of patients with focal epilepsy unfortunately either start as or become refractory to medical management and the only alternative is neurosurgical resection of the focus that often leaves patients with neurologic deficits. Previous work utilizing two-photon imaging of mice showed that cells in the supragranular layers of the neocortex are consistently activated first during horizontal seizure propagation. Therefore, it has been theorized that these lateral connections could be severed to stop seizure propagation while maintaining other connections to sustain neural function. Using tightly-focused femtosecond duration laser pulses, we can now make micrometer sized cuts deep below the surface of the brain without damaging the surrounding tissue. We tested the long-term efficacy of laser cuts in layers II-IV surrounding a chronic neocortical seizure focus as well as determined the effect these cuts have on neuronal structure and sensory-motor function. Chronic focal neocortical seizures were initiated through a microinjection of iron chloride. Control animals had 85% of seizures propagate while mice with laser cuts had only 5% of seizures propagate. Seizure metrics including duration, max band power, and area under the curve at the seizure focus showed that non-propagated seizures in mice with laser cuts were indistinguishable from propagated seizures in control animals, indicating that without laser cuts these seizures would have propagated. Laser cuts themselves only caused minor structural changes including a small scar with slight gliosis at one month and an acute drop in blood flow at the cut border that recovered within a week. To determine the effect of laser cuts on normal function we localized laser cuts to forelimb motor cortex and saw only minor acute deficits in a skilled reaching task that returned to normal within days. Ultimately, this laser scalpel approach was effective in blocking seizures and caused negligible structural and functional deficits indicating that it could be a viable approach for future treatment of drug-resistant focal neocortical epilepsy.

Disclosures: S. Lieberman: None. **D.A. Rivera:** None. **R. Morton:** None. **A. Hingorani:** None. **T. Southard:** None. **L. Johnson:** None. **J. Reukauf:** None. **R.E. Radwanski:** None. **M. Zhao:** None. **N. Nishimura:** None. **O. Bracko:** None. **T.H. Schwartz:** None. **C.B. Schaffer:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.01

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

Support: NIH Grant NS090934
NIH Grant AG047644

Title: The relationship between ApoE and lipid dysregulation in glia of the PS19 mouse model of tauopathy.

Authors: *A. LITVINCHUK[1], K. LIN[2], N. BIEN-LY[2], H. P. BENTON[2], S. DAVIS[2], J. SUH[2], J. D. ULRICH[1], G. DI PAOLO[2], D. M. HOLTZMAN[1];
[1]Dept. Neurol., Washington Univ. in St. Louis, Saint Louis, MO; [2]Denali Therapeut., San Francisco, CA

Abstract: Emerging evidence suggests that the accumulation of certain lipids in glia is linked to enhanced inflammatory responses and, thus, could significantly promote neurodegeneration in the context of Alzheimer`s disease (AD). Apolipoprotein E (apoE) plays an essential role in brain lipid homeostasis; furthermore, the presence of the apoE4 allele is the strongest genetic risk factor for developing late-onset AD compared to most common apoE3 and protective apoE2 alleles. Several studies detect lipid droplets in cultured human apoE4 iPSC-derived astrocytes and microglia; transcriptomic and lipidomic profiling of these cells reveals upregulation of cholesterol/matrisome and inflammatory pathways. Additionally, recent studies documented an increase in free cholesterol and cholesteryl esters in microglia of apoE KO mice following demyelination that led to enhanced inflammasome activation. Therefore, apoE may modulate cellular toxicity through regulation of lipid pathways and inflammation. However, it is not known if lipid accumulation in glia mediates toxic effects in vivo in the context of tauopathy. Here, we utilized the P301S tau transgenic mice with a targeted replacement of murine apoE with human ApoE4, ApoE3, or ApoE KO to dissect the role of apoE isoforms in glial lipid metabolism. We demonstrate that microglia from aged P301S/ApoE4 mice accumulate a significant amount of large BODIPY-positive lipid inclusions within lysosomes compared to control mice. By performing unbiased FACS coupled with lipidomics of forebrain samples and sorted microglia and astrocytes from ApoE KO, ApoE KI and P301S/ApoE KI mice, we demonstrate that microglia accumulate significant amounts of specific cholesteryl esters, sphingolipids and diacyl glycerols in an ApoE-isoform and Tau-dependent manner. These changes could contribute to impaired lysosomal function, defects in lipid clearance and subsequent neuroinflammation observed in aged tauopathy mice. We also show significant changes in levels of specific cholesteryl esters in sorted astrocytes that occur in an Apoe-dependent manner but are not further affected by tau pathology. Finally, by performing lipidomics on bulk forebrain samples, we detect altered levels of plasmalogens and BMPs in P301S/ApoE KI mice compared to control groups indicating that other cell types could also contribute to regulation of lipid homeostasis in brains of aged tauopathy mice. Together, we demonstrate that ApoE4 promotes lipid accumulation in glia in the setting of tauopathy. We hypothesize that reducing the accumulation of some of these lipid species, particularly in microglia, could be anti-inflammatory and neuroprotective.

Disclosures: A. Litvinchuk: None. **K. Lin:** A. Employment/Salary (full or part-time):; Employee, Denali Therapeutics. **N. Bien-Ly:** A. Employment/Salary (full or part-time):; Employee, Denali Therapeutics. **H.P. Benton:** A. Employment/Salary (full or part-time):; Employee, Denali Therapeutics. **S. Davis:** A. Employment/Salary (full or part-time):; Employee, Denali Therapeutics. **J. Suh:** A. Employment/Salary (full or part-time):; Employee, Denali Therapeutics. **J.D. Ulrich:** None. **G. Di Paolo:** A. Employment/Salary (full or part-time):;

Employee, Denali Therapeutics. **D.M. Holtzman:** A. Employment/Salary (full or part-time):; Co-founder, C2N Diagnostics LLC. F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Takeda, Casma, Eli Lilly.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.02

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

Support: VA Merit Award 101 BX004161-01
NIH R01 ES028104
NIH R01 ES029835GW

Title: Ozone air pollution exposure impairs plaque-associated microglia and alters the peri-plaque microenvironment through a bi-directional lung-brain axis

Authors: *H. J. GREVE[1], C. GARZA LOMBO[1], A. L. DUNBAR[1], C. AHMED[1], M. THANG[1], E. J. MESSENGER[1], C. L. MUMAW[1], J. A. JOHNSON, Jr.[1], U. P. KODAVANTI[3], A. L. OBLAK[2], M. L. BLOCK[1];
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Abstract: An increasing body of epidemiological reports implicates components of outdoor air pollution, such as ozone (O_3), as a risk factor for Alzheimer's disease (AD). However, the mechanisms underlying this increased risk with O_3 are poorly understood. Due to its reactive chemistry, O_3 is unable to pass beyond the respiratory tract to directly interact with the brain parenchyma, pointing to an important role for O_3-induced peripheral changes in triggering CNS effects. The Lung-Brain Axis hypothesis holds that pulmonary damage from inhaled pollutants, such as O_3, causes circulating signals to augment CNS disease, particularly AD, but the underlying mechanisms remain largely unexplored. To begin to address these issues, 8-10-week-old male 5xFAD mice, a model of AD, and littermate control animals were exposed to O_3 (0, 0.3, 1.0 ppm) for 13 weeks (4 hours/day, 3 days/week). Thioflavin S staining in the brains of 5xFAD mice showed a dose-dependent increase in the number of plaques in the cortex and hippocampus of the 1.0 ppm O_3 exposed group, demonstrating that subchronic O_3 exposure augments amyloid neuropathology. Analysis of the plaque microenvironment showed decreased microglial-plaque association without a change in total number of microglia, suggesting that O_3 may perturb the microglia plaque response. Further, using spatial proteomics through Nanostring's GeoMX DSP platform, an O_3-induced dysregulated protein expression was observed in both the peri-plaque

microenvironment as well as in plaque distant regions of the cortex, suggesting that O_3 exposure dysregulates the CNS response to amyloid pathology at the level of the peri-plaque microenvironment. Specifically, protein expression of microglial markers (Clec7a, P2rx7, and MerTK), Aβ processing proteins (BACE1, ADAM10, IDE), phosphor-tau, and damage and immune markers (S100b, MBP, CD169) were altered in O_3-exposed brains. Analysis of the pulmonary response of 5xFAD and WT mice to O_3 showed an increase in total cells, macrophages, and neutrophils in the bronchoalveolar lavage (BAL) of 5xFAD mice, but not WT controls. Taken together, these data support that subchronic O_3 exposure, a model of urban air pollution, exacerbates AD-like neuropathology, impairs microglia-plaque association, dysregulates protein expression at the level of the peri-plaque microenvironment, and that ongoing neurodegenerative pathology may regulate pulmonary and peripheral immune response to O_3, which has crucial implications for how air pollution my affect neurodegenerative disease.

Disclosures: H.J. Greve: None. **C. Garza Lombo:** None. **A.L. Dunbar:** None. **C. Ahmed:** None. **M. Thang:** None. **E.J. Messenger:** None. **C.L. Mumaw:** None. **J.A. Johnson:** None. **U.P. Kodavanti:** None. **A.L. Oblak:** None. **M.L. Block:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.03

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

Support: GMU Oscar Grant

Title: Potential Neuroinflammatory Benefits of Low-dose Naltrexone and the Development of Alzheimer’s Disease in h-tau Mouse Models

Authors: *S. NEFF[1], J. O. SANCHEZ[1], A. BOOTH[1], M. U. ALI[1], A. VILLATORO[1], J. M. FLINN[2];
[2]Psychology, [1]George Mason Univ., Fairfax, VA

Abstract: Rates of Alzheimer’s Disease (AD) among American adults are predicted to increase by 22% between 2020 and 2025, and is being called a public health crisis by the CDC. The underlying pathology marking the changes in behavior and cognition seen in AD are the accumulation of amyloid plaques and tau tangles, and research has suggested a role of neuroinflammation in the development of AD. One promising treatment, low-dose Naltrexone (LDN), has the potential to modulate inflammatory pathways in the brain involved in the development of AD. Using the rTg4510 h-tau mouse model with wildtype (WT) mice as a control, we began administering LDN or Phosphate-buffered saline (PBS) by intraperitoneal injection beginning at 2 months, every other day for 6 weeks. We tested 4 groups, including WT

LDN (n = 12), h-tau LDN (n = 12), WT PBS (n = 12), h-tau PBS (n = 12). Naltrexone was diluted by 0.1 mg Naltrexone per 1 kg water, and the amount of LDN administered to each mouse was based on body weight at the time of injection at 1 ml per 1 g. The mice not receiving LDN injections received 20 ml PBS injections. Mice were tested at 3.5 months of age for spatial memory deficits using the Morris Water Maze (MWM) behavioral testing method. ANOVA showed significant results of genotype, with WT mice performing significantly better than h-tau mice ($p < .001$) for latency to platform. There were no statistically significant differences between LDN and PBS injection types ($p > .05$) for latency. Additional behavioral tests and Western Blot analyses will be performed.

Disclosures: S. Neff: None. **J.O. Sanchez:** None. **A. Booth:** None. **M.U. Ali:** None. **A. Villatoro:** None. **J.M. Flinn:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.04

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

Support: Washington University in St. Louis Knight ADRC T32

Title: Astrocytic BMAL1 regulates protein aggregation in mouse models of alpha-synuclein and tau pathology

Authors: *P. W. SHEEHAN, M. KANAN, C. NADARAJAH, J. PATTERSON, B. BENITEZ, O. HARARI, L. BRASE, A. A. DAVIS, E. S. MUSIEK;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: The circadian clock regulates inflammatory responses in the peripheral immune system, though its function in neuroinflammation is poorly understood. Deletion of the master circadian clock transcription factor BMAL1 abrogates cellular circadian clock function and can be used to probe cell-type specific functions of the clock. Our lab has previously shown that *Bmal1* deletion induces oxidative stress, astrocyte activation, and increased β-amyloid plaque deposition in mice. Given these findings, we hypothesized that deletion of *Bmal1* would increase pathology in other protein aggregation models. Therefore, we tested the effects of *Bmal1* deletion in a model of alpha-synuclein (aSyn) aggregation and spreading using a preformed fibril (PFF) injection model. Unexpectedly, we found that global *Bmal1* deletion significantly decreased aSyn spreading throughout the brain. Because *Bmal1* deletion drives cell-autonomous astrocyte activation, we examined aSyn pathology in astrocyte-specific *Bmal1* knock out mice (Aldh1l1-Cre^{ERT2};Bmal$^{f/f}$) and found that deletion of *Bmal1* in astrocytes was sufficient to decrease aSyn pathology. Furthermore, we generated novel mouse lines to test the loss of *Bmal1*

on tau aggregation. We found that global *Bmal1* deletion strongly mitigates tau pathology, an effect that was once again recapitulated using astrocyte-specific *Bmal1* knock out mice. We observed that astrocytic *Bmal1* deletion rapidly induced astrocyte-specific expression of BAG3, a chaperone protein previously implicated in the degradation of aggregated tau and synuclein. To test whether BAG3 overexpression might be the mechanism driving the decreased protein aggregation observed in astrocyte *Bmal1* knockout mice, we developed an *in vitro* astrocyte PFF uptake assay and found that loss of *Bmal1* increased PFF uptake, which was reduced to baseline after simultaneous knockdown of *Bag3*. To investigate the relevance of BAG3 in human disease, we looked at a single nucleus RNA-sequencing database of patients with autosomal dominant Alzheimer's Disease (ADAD). Interestingly, we found that BAG3 was specifically upregulated in activated astrocytes in patients with ADAD. Ongoing studies are examining the effect of BAG3 knockdown on aSyn spreading in astrocyte-specific *Bmal1* KO mice. Overall, these findings suggest that early astrocyte activation via *Bmal1* deletion prevents neurodegenerative protein aggregation, possibly through the upregulation of BAG3. This novel data suggests that targeting astrocytic protein degradation machinery could be a therapeutic strategy for mitigating Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases.

Disclosures: P.W. Sheehan: None. **M. Kanan:** None. **C. Nadarajah:** None. **J. Patterson:** None. **B. Benitez:** None. **O. Harari:** None. **L. Brase:** None. **A.A. Davis:** None. **E.S. Musiek:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.05

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

Support: T32AG05851804
Good Ventures
NS090934

Title: Elucidating the effect of the antibiotic altered microbiota on amyloid deposition as influenced by TREM2 in mouse model of amylodosis.

Authors: *M. E. BOSCH, A. NAMBIAR, H. DAVID, S. PARHIZKAR, D. HOLTZMAN; Washington Univ., St. Louis, MO

Abstract: Alzheimer disease (AD) is the most common neurodegenerative disease that causes progressive memory loss and cognitive decline in people over the age of 65. Longitudinal biomarker studies indicate AD pathology begins in a preclinical phase when Aβ accumulates over 15-20 years prior to the onset of cognitive dysfunction. The preclinical phase affords the

opportunity to identify alterations in pathways that develop before the onset of symptoms that could provide important insight into mechanisms underlying disease progression. One such pre-clinical symptom could be microbiome dysbiosis. The microbiome has many vital homeostatic functions including regulating immune functions and neurological development. Multiple studies have shown that AD patients have a significantly different microbiome composition compared to healthy, age-matched controls. Interestingly, when the microbiome is altered in mouse models that develop Aβ deposition either by antibiotics or complete ablation, there is a significant reduction of Aβ deposition, improved memory function, decreased neuronal loss, and reduced microglial neuroinflammation. These studies demonstrate that alterations in the gut microbiome are connected to AD pathology and may be linked with systemic inflammation resulting in prolonged neuroinflammation and disease progression. Recent genome-wide association studies found mutations within microglia TREM2 (e.g. R47H) increases the risk of developing late-onset AD by 2-4 fold. Our lab has published that loss of TREM2 function in models with Aβ deposition causes' increased Aβ deposition and decreased plaque-associated microglia leading to increased dystrophic neurites around plaques. The exact mechanism of how TREM2 contributes to AD progression is unknown. Therefore it was hypothesized that altering the microbiome via antibiotic treatment in amyloid-depositing mice will improve microglial Aβ recognition and reduce plaque burden in R47H amyloid depositing mice. To test this, R47H or WT mice were gavaged with antibiotics or vehicle daily from P14 to P21. At 4.5 months old, antibiotic treated mice had larger cecum weights signifying a disruption in gut flora. Initial results indicate that antibiotic treatment reduces Aβ plaque size in R47H AD mice compared to controls. Antibiotic treatment also enhanced microglia clustering around amyloid plaques and decreased microglia branch length in R47H AD mice compared to controls indicating an alteration in microglia activation state. These data suggest that altering the microbiome has the potential to impact Aβ-linked pathology via microglia.

Disclosures: M.E. Bosch: None. **A. Nambiar:** None. **H. David:** None. **S. Parhizkar:** None. **D. Holtzman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N Diagnostics LLC. F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Takeda, Casma, Eli Lilly.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.06

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

Support: NIH Grant R01NS105971
NIH Grant R01NS093362

The Bluefield Project to Cure Frontotemporal Dementia
BrightFocus Foundation

Title: Proteomics connects microglial GPNMB upregulation to neuroinflammation and neurodegeneration in a mouse model of frontotemporal dementia caused by progranulin deficiency

Authors: *T. KUKAR[1], P. MERINO[1], G. TAYLOR[1], M. WANG[1], M. HUANG[2];
[1]Dept. of Pharmacol. and Chem. Biol., Emory Univ., Atlanta, GA; [2]Second Xiangya Hospital, Xiangya Sch. of Med., Central South Univ., Changsha, China

Abstract: Mutations in the *GRN* gene, encoding progranulin (PGRN), are a common cause of Frontotemporal dementia (FTD) and lead to PGRN haploinsufficiency. PGRN is composed of 7.5 repeating domains termed granulins (GRNs 1-7) that are joined by linker regions. We have discovered that PGRN is processed into ~6 kDa granulins within the lysosome, where we hypothesize they are critical for lysosome homeostasis (Holler et al, *eNeuro*, 2017 Aug 18;4(4)). However, the molecular mechanisms caused by decreased lysosomal PGRN/granulin levels that lead to FTD, Alzheimer's disease (AD), and other neurodegenerative disorders are still unknown. To gain insight into the mechanisms of FTD pathogenesis, we performed an unbiased quantitative proteomic analysis of whole-brain tissue from wild-type ($Grn^{+/+}$) and progranulin knockout ($Grn^{-/-}$) mice at 3 and 19-months of age using Tandem-Mass-Tag (TMT) isobaric labeling mass spectrometry. In 19-month-old $Grn^{-/-}$ mice, 119 proteins were increased, and 20 proteins were decreased compared to $Grn^{+/+}$ mice. The most significantly upregulated protein in the brains of 19-month-old $Grn^{-/-}$ mice was Glycoprotein NMB (GPNMB), motivating us to investigate GPNMB in more detail. GPNMB is a type-I transmembrane glycoprotein of unknown function in the brain. We find GPNMB is predominantly expressed by microglia and strongly upregulated in $Grn^{-/-}$ mice with age. Importantly, microglia in brains of human FTD-*GRN* cases also upregulate GPNMB. Recently, GPNMB was found to be upregulated by microglia surrounding amyloid plaques in AD. Moreover, upregulation of GPNMB is a marker of degenerative disease-associated microglia (DAM). Additionally, genetic variants in *GPNMB* are associated with Parkinson's disease. Thus, GPNMB is broadly involved in neurodegeneration and may represent a novel drug target. However, it is unclear whether upregulation of GPNMB in microglia is protective or deleterious. Because overexpression of GPNMB is deleterious in several diseases, we hypothesize that microglial upregulation of GPNMB in $Grn^{-/-}$ mice activates pro-inflammatory pathways that contribute to neurodegeneration. We are currently testing this hypothesis, by crossing $Grn^{-/-}$ and $Gpnmb^{-/-}$ mice and determining how elimination of GPNMB alters lysosome dysfunction, inflammation, and neurodegeneration in a mouse model of progranulin deficiency. Data from these experiments will: a) help decipher the role of microglial expression of GPNMB in mediating inflammation and neurodegeneration caused by loss of PGRN and granulins and b) provide insight whether targeting GPNMB is a novel, viable therapeutic approach.

Disclosures: T. Kukar: None. **P. Merino:** None. **G. Taylor:** None. **M. Wang:** None. **M. Huang:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.07

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

Support: DFG Research Fellowship
Pacific Alzheimer Research Foundation grant

Title: Neuronal oxidative stress at amyloid plaques is not caused by microglia but is correlated with dysfunctional lysosome accumulation

Authors: *S. WENDT, B. A. MACVICAR;
Ctr. for Brain Health/Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Intense oxidative stress occurs at Alzheimer's Disease (AD) plaques contributing to synaptic dysfunction and neuronal death yet the triggers for generating reactive oxygen species (ROS) remain elusive. Microglia are thought to contribute to AD pathology and to modify ROS generation by acquiring neuroprotective or neurotoxic phenotypes. Here we report new imaging strategies using genetically encoded roGFP redox sensors to simultaneously image microglia and neuronal redox states in live cells in vivo and in paraformaldehyde fixed brain tissue with additional immunocytochemistry. By employing these tools, we found that microglia preferentially surround and engulf dystrophic neurites (DNs) with the greatest degree of cytoplasmic oxidation around amyloid plaques in 5xFAD mice. High levels of cytoplasmic oxidation in DNs correlated with elevated LAMP1 and ubiquitin thereby linking dysfunctional lysosomes to oxidative stress in DNs. Long-term in vivo 2-photon imaging revealed highly dynamic interactions between microglia and DNs. When microglia were depleted from 5xFAD mice by administering PLX3397, oxidative stress surprisingly was exacerbated which correlated with even greater elevations of LAMP1. Interestingly, we also confirmed the previously reported neuroprotective effect of microglia depletion in 5xFAD mice and found reduced levels of neuronal loss after treatment. Our data highlights two seemingly paradoxical functions of microglia in 5xFAD mice: While microglia ameliorate redox stress in DNs they also promote the loss of neurons. Our novel approaches to study microglia and neuronal redox levels simultaneously allow to further dissect the underlying redox mechanisms and identify key pathways of neurotoxic and neuroprotective microglial functions in AD.

Disclosures: S. Wendt: None. **B.A. MacVicar:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.08

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

Support: NIH Grant HL142752

Title: Gestational intermittent hypoxia sex-dependently alters cognitive function in 5xFAD Alzheimer's mouse model

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Abstract: The in utero environment is well known to influence CNS development and behavioral phenotypes, effects that can last into adulthood. However, long-term cognitive reprogramming has never been evaluated in the context of sleep-disordered breathing (SDB) during pregnancy, despite its alarming increase in recent years and detrimental consequences. Alzheimer's Disease (AD) among other neurocognitive disorders, has been observed to be strongly correlated with sleep apnea, a manifestation of SDB. Here, we examined the 5XFAD Alzheimer's mouse model after in utero exposure to gestational intermittent hypoxia (GIH), a hallmark of SDB during pregnancy. We tested the hypothesis that GIH exposure will compound the cognitive and behavioral phenotypes previously observed in 5xFAD mice to reveal further deficits. Pregnant dams received intermittent hypoxia (2 min alternating 6.5%/21% O2) or normoxia (2 min alternating 21% O2/21% O2) for 12 hrs/day from gestational days 10-18. Offspring were then grown up to 4 mo, at which point behavioral tests such as marble burying, Y-maze, and novel object recognition were conducted every two months across the lifespan to 8 months. Preliminary behavioral data demonstrated sex-dependent declines in cognitive function of 5xFAD with exposure to GIH. After final behavior testing, brains were harvested for immunohistochemistry analysis. Imaging analysis demonstrated sexually dimorphic alterations in microglial immune response with exposure to GIH. These data support sex-specific alterations in cognitive function in AD mice based on early life exposures.

Disclosures: P.R. Romero: None. **A.C. Ewald:** None. **K.M. Marino:** None. **A.B. Radcliff:** None. **E.C. McCann:** None. **T.K. Ulland:** None. **T.L. Baker:** None. **J.J. Watters:** None.

Poster

P204. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P204.09

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

Support: NIH [1R01AG057555 (to L.X. (PI), M.E.F.-P., P.R., P.S.)]
NIH [5T32HL135465-03 (C.H.W. fellowship)]
The Graduate Center (Biochemistry program) at City University of New York

Title: Altered prostaglandin D2 signaling via its hippocampal DP1 and DP2 receptors in a transgenic rat model of Alzheimer's disease

Authors: *C. WALLACE[1,3], L. XIE[2], P. ROCKWELL[1], P. A. SERRANO[4], M. E. FIGUEIREDO-PEREIRA[1];
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Abstract: Alzheimer's Disease (AD) is the most common neurodegenerative disease that disproportionately affects an aged population. AD exhibits a wide array of pathologies including amyloid plaques, neurofibrillary tangles, neuroinflammation, neuronal loss, brain atrophy, and cognitive decline. Many efforts to treat the disease rely on targeting plaques or tangles, but there is still much to learn about AD in its other pathologies. Epidemiological evidence points to the use of over-the-counter NSAIDs such as aspirin or ibuprofen, but clinical trials fail to show a significant benefit. A deeper investigation is required into the pathway that NSAIDs target which is the cyclooxygenase (COX) pathway. The COX pathway is controlled by prostaglandins which have multiple receptors and many functions including inflammation, nociception, sleep, reproduction, and vascular function. In the brain, prostaglandin D2 (PGD2) is the most abundant prostaglandin, the one that increases most under pathological conditions, and plays roles in sleep and inflammation. PGD2 signals through the DP1 and DP2 receptors and their activation will either raise or lower intracellular cAMP levels, respectively. We addressed the role of PGD2 signaling in AD with Fischer transgenic 344-AD (TgF344) rats that express the AD related human genes "Swedish" amyloid-precursor protein (APPswe) and Δ exon 9 presenilin-1 (PS1ΔE9). TgF344 rats exhibit age-dependent amyloid plaques, tau tangles, neuronal loss, microgliosis, and cognitive decline. Our imunohistochemical analysis of hippocampal tissue found that DP2 is detected mostly in neurons. Moreover, TgF344 rats exhibited higher DP2 levels in neurons of the CA1 region than their wild type (WT) littermates. In contrast, DP1 was expressed in microglia. TgF344 rats also exhibited higher levels of DP1 in microglia of the CA1 (stratum lacunosum moleculare) region and DG (hillar) region than their WT littermates. Our cognitive behavioral analyses using two hippocampal-dependent assays, the 8-arm radial arm maze and the active place avoidance test, established that WT rats performed significantly better than the TgF344 rats. Our findings demonstrate that the levels of DP1 and DP2 receptors are altered in TgF344 rats specifically within the CA1 and DG regions of the hippocampus. Thus, modulating the activity of these receptors could help dampen AD pathology and offer a new therapeutic strategy.

Disclosures: C. Wallace: None. **L. Xie:** None. **P. Rockwell:** None. **P.A. Serrano:** None. **M.E. Figueiredo-Pereira:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ARO W911NF-15-1-0432
NIH 5R25GM077634

Title: Modulated integrin signaling plays a role in anticholinesterase-induced synaptopathy in hippocampal explants

Authors: J. J. TUTON, M. F. ALMEIDA, B. A. BAHR, *K. L. G. FARIZATTO;
Biotech. Res. and Training Ctr., Univ. of North Carolina - Pembroke, Pembroke, NC

Abstract: Organophosphates (OPs), a deadly class of poisons which inhibit acetylcholinesterase, lead to cholinergic crises and produce the risk of excitotoxic injuries (e.g. seizure pathology), long-term neurological problems, and death. OPs are used worldwide for agricultural pesticides and as warfare agents used by terrorist groups and state actors. Understanding the synaptoxic profile of OPs is vital for public health and long-term treatment strategies. OP-mediated synaptopathology has been described (Farizatto et al. 2017 *J Mol Neurosci* 63:115; Farizatto et al. 2019 *Sci Rep*.9:6532), with the latter study identifying an involvement by integrin-type adhesion responses. From *C. elegans* to the mammalian brain, integrins are linked to synaptogenesis, synaptic modulation, and axon regeneration (Bahr 2000 *J Neurosci Res* 59:827; Eva & Fawcett 2014 *Curr Opin Neurobiol* 27:179; Hisamoto et al. 2018 *Nat Commun* 9:3099). Here, hippocampal tissue cultures were exposed to the OP toxin paraoxon (Pxn) to decipher if the evident synaptic vulnerability is influenced by integrins, using the β1-integrin inhibitor (BIO 5192). Additionally, a set of treated hippocampal slices were subsequently followed with a toxin washout procedure. Synaptic changes were assessed by immunoblot, immunohistochemistry, and correlational analyses. Pxn exposure caused a dramatic loss of pre- and postsynaptic staining among neuropilar dendrites, occurring in correspondence with abnormal induction of adhesion responses through theβ1 integrin. Such an increase in β1 integrins was evident across the hippocampal dendritic fields. Adhesion responses are mediated by multiple types of complex adhesion molecules. Interestingly, in the Pxn exposure model, the distinctive adhesion response was mediated solely by the β1-typeintegrin family when assessed in parallel with neurexin and NCAM. To further examine the integrin's role during OP-mediated synaptopathology, we co-treated the Pxn-exposed tissue with a neuroprotective trifluoromethylketophenoxypentyl analog, and observed that the integrin-type response was offset by the neuroprotectant which also helped stabilize synaptic markers. Similar results were found with the toxin wash-out procedure. Additionally, the synaptic compromise observed in Pxn-exposed explants was not exhibited after the application of the β1-integrininhibitor, suggesting that blocking β1 integrin signaling appears to attenuate the unique synaptotoxicity mediated by Pxn. These findings postulate that integrins

can respond to excitotoxic insults and induce distinct cell signaling that influences synaptic integrity and maintenance.

Disclosures: J.J. Tuton: None. **M.F. Almeida:** None. **B.A. Bahr:** None. **K.L.G. Farizatto:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Project Grant PJT 153319
Dalhousie Medical Research Foundation Durland Breakthrough Fund
Dalhousie Medical Research Foundation DMRF Irene MacDonald Sobey Chair in Curative Approaches to Alzheimer's Disease
Alzheimer's Society Research Program and Canadian Consortium on Neurodegeneration in Aging Master's Scholarship
Dalhousie Faculty of Medicine Studentship
Dalhousie Medical Research Foundation DMRF Weismann Canada Studentship in Medical Neuroscience

Title: Cholinergic Preservation as a Mechanism of Resilience in the Oldest-Old

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Abstract: Background: A subpopulation of the oldest-old, individuals 80 years of age and older, maintain cognitive abilities comparable to individuals who are decades younger. Both the rostral prefrontal cortex (rPFC) and hippocampus are implicated in cognitive function mediated, in part, by cholinergic innervation via the basal forebrain. The aim of this study is to evaluate the deposition of neuropathological proteins in the oldest-old rPFC and hippocampus and evaluate cholinergic system preservation as a potential mechanism of cognitive resilience. **Methods:** Human formalin-fixed rPFC and hippocampal tissues from male and female cognitively normal oldest-old (CNOO) individuals over the age of 80 were examined and compared to sex- and age-matched Alzheimer's disease tissues. Neuropathological load was examined with immunohistochemical staining for β-amyloid (Aβ), tau, α-synuclein, and phosphorylated Tar DNA-binding protein-43 (TDP-43). The presence of cholinergic dysfunction was evaluated using histochemical staining for butyrylcholinesterase (BChE) and acetylcholinesterase, and immunohistochemical staining for choline acetyltransferase (ChAT). Neuropathological findings

were analyzed using a modified CERAD neuropathological protocol. ChAT was analyzed qualitatively. **Results:** The rPFC and hippocampus had significant deposition of abnormal protein aggregates characteristic of neurodegeneration. While cholinesterase-positive plaques were observed in the regions examined in the CNOO cases, cholinesterase-positive plaque deposition was greater in AD cases, indicating a specificity to AD-associated pathology. ChAT activity was normal in the CNOO and reduced in the AD rPFC and hippocampus. **Conclusion:** The CNOO maintain cognitive function despite significant neuropathological burden. Maintained cholinergic connectivity to the rPFC and hippocampus may contribute to cognitive resilience in the CNOO.

Disclosures: S.P. Maxwell: None. **M.K. Cash:** None. **S. Darvesh:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Human iPSC microglia for assessing new treatments in neurological diseases

Authors: *F. SIMON, K. WALKER, J. BELLEC-DYEVRE, C. CANCIO, M. PAQUET, E. ESNEAULT;
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Abstract: Microglial activation, and the ensuing inflammatory response in the central nervous system, plays an important role in neurodegenerative diseases such as Alzheimer's and Parkinson's. Activated microglia, in addition to their role in phagocytosis, may induce the secretion of pro-inflammatory cytokines, including TNF-α and Il-6, as well as caspase 1 activation via the NLRP3 inflammasome, which controls the release of Il-1beta and Il-18.
The aim of the present study was to assess functional and physiological properties of microglia derived from human induced pluripotent stem cells (human iPSC) by measuring the expression of proinflammatory cytokines secreted following LPS stimulation and their ability to phagocytose bacterial particles.
Expression levels of IL-6, IL-8, IL-10 and TNF-α were clearly increased in the supernatants at 6 and 24 hours following LPS stimulation, demonstrating microglial activation. These effects were partially inhibited by Dexamethasone, a synthetic glucocorticoid, used for its anti-inflammatory properties. The addition of an adenosine triphosphate pulse following LPS was required to induce Il-1 beta secretion, confirming the activation of the inflammasome pathway
Microglia functionality was assessed via kinetic monitoring of the phagocytosis of bacterial particles, coupled with pH-Rodo fluorochrome. An increase of fluorescent signal was observed over time, and activated microglia exhibited functional phagocytosis. This effect was fully

inhibited in presence of Cytochalasin D, an inhibitor of actin polymerization.
These results suggest that human iPSC-derived microglia exhibit functional characteristics similar to human microglia, including phagocytosis and cytokine-mediated inflammatory responses. This translational model may be used to study neuroinflammation, a crucial hallmark of neurodegenerative disorders in humans, and to assess the efficacy of early-stage therapeutic targets.

Disclosures: F. Simon: None. **K. Walker:** None. **J. Bellec-Dyevre:** None. **C. Cancio:** None. **M. Paquet:** None. **E. Esneault:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS109252

Title: Mitochondria-lysosome contact regulation and function in neurodegenerative diseases

Authors: *J. CISNEROS, G. C. SHUM, Y. C. WONG;
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Abstract: Multiple neurodegenerative diseases including Parkinson's disease and Charcot-Marie-Tooth disease have been genetically and functionally linked to defects in mitochondria and lysosomes, suggesting that proper regulation of these two organelles is critical for maintaining neuronal homeostasis. Importantly, inter-organelle contacts form between mitochondria and lysosomes, allowing for their crosstalk at dynamic mitochondria-lysosome contacts sites. Using advanced live cell confocal microscopy, we have examined how mitochondria-lysosome contact sites are dynamically regulated and their roles in regulating mitochondrial and lysosomal functions. Mitochondria-lysosome contacts mediate the bidirectional regulation of both mitochondrial and lysosomal network dynamics, and are further regulated by multiple protein complexes on both organelles. Furthermore, mitochondria-lysosome contact regulation and functions are disrupted by mutations in various neurodegenerative diseases including both Parkinson's disease and Charcot-Marie-Tooth disease. These findings further support a key role for this pathway in regulating neuronal homeostasis, and in potentially driving the etiology of multiple neurological disorders associated with mitochondrial and/or lysosomal dysfunction.

Disclosures: J. Cisneros: None. **G.C. Shum:** None. **Y.C. Wong:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P51-OD011107-57S3
NIH Grant P51-OD011107-58S2

Title: Deployment of a non-human primate (NHP) model to study the intersection between Alzheimer's disease (AD) and adult hippocampal neurogenesis

Authors: *G. B. DINIZ[1], D. BECKMAN[1], S. OTT[1], P. CHAKRABARTY[3], S. MULLER[4], J. H. KORDOWER[4,5], M. G. BAXTER[6,7], J. MORRISON[1,2];
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Abstract: New neurons are generated in the hippocampus past the developmental period and childhood well into adult life, a process that has been extensively investigated in rodents, but that remains controversial in humans. The hippocampus is also notable for its vulnerability in Alzheimer's disease (AD), as pathological markers are observed in this structure early during the course of the disease. Many of the operational functions affected by AD depend at least partially on proper hippocampal function, and multiple animal and histopathological studies indicate that adult hippocampal neurogenesis is affected during AD. However, the exact mechanisms of interaction between these two processes remain poorly elucidated, particularly due to the inadequate translation of rodent animal model-derived knowledge to both the dementia and neurogenesis fields. In light of the above, we propose the use of non-human primate models (*rhesus* macaques) recently developed by our group - including the injection of synaptotoxic amyloid β oligomers (AβOs) in the cerebroventricular space and the viral induction of tau expression in the entorhinal cortex - to understand the effects of AD-related proteins over the progenitor and proliferating populations of the primate hippocampus. To accomplish those goals, we will employ modern immunohistochemistry, microscopy, and segmentation and reconstruction techniques, allowing us to visualize the different components of the neurogenic niche and the effects of aging, amyloid, and tau proteins over those components in a highly controlled setting. Our preliminary data suggest a substantial and selective decrease in the number of immature neurons in the granular zone of the dentate gyrus ($Tuj1^+$) of adult macaques injected with synthetic AβOs compared to scramble peptide controls. The magnitude of this decrease was similar to that observed between aged and young animals. At the same time, exogenous tau expression has minimal effect on the number of $Tuj1^+$ neurons compared to

uninfected controls, despite the abundant presence of phosphorylated tau in the dentate gyrus and the underlying subgranular zone. Our results suggest that amyloid oligomers are capable of driving neurogenic deficits in AD even in the absence of tau aggregation, with neurogenic deficits possibly accompanying the synaptic dysfunction promoted by amyloid early in the course of the disease. Therefore, early pharmacological intervention against amyloid pathology may be a valuable strategy to preserve adult hippocampal neurogenesis in physiological and pathological situations.

Disclosures: G.B. Diniz: None. **D. Beckman:** None. **S. Ott:** None. **P. Chakrabarty:** None. **S. Muller:** None. **J.H. Kordower:** None. **M.G. Baxter:** None. **J. Morrison:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant # HL146832

Title: Fibrinogen-induced oxidative stress in neurons

Authors: *N. SULIMAI, J. BROWN, D. LOMINADZE;
Univ. of South Florida, Tampa, FL

Abstract: Traumatic brain injury (TBI) is associated with systemic inflammation accompanied with an elevated level of fibrinogen (Fg) and increased cerebrovascular permeability, which could result in the translocation of Fg from non-ruptured vessels to the extravascular space. We previously found that extravasated Fg was deposited between blood vessels and astrocyte endfeet. This effect was coincided with increased neurodegeneration and a reduction in short term memory (STM) during mild-to-moderate TBI (m-mTBI). We also showed that the association of Fg with astrocytic intercellular adhesion molecule-1 (ICAM-1) and cellular prion protein (PrP^C) caused astrocyte activation, expression of pro-inflammatory cytokines, production of reactive oxygen species (ROS), and astrocyte death. We aimed to investigate if Fg can directly interact with neurons via its neuronal receptors ICAM-1 and PrP^C and if this effect can cause oxidative stress affecting neuronal viability. Primary mouse brain cortical neurons from C57BL/6 mice were grown on Poly-D-Lysine and laminin-coated glass-bottomed 24-well or 8-well chambers at the density of 200,000 cells/well. Neurons were treated with various concentrations of Fg or with Fg in the presence of function-blockers of its receptors ICAM-1 or PrP^C for 3 or 24 hours. All groups contained the thrombin inhibitor hirudin to prevent conversion of Fg into fibrin. Associations of Fg with neuronal ICAM-1 and PrP^C were characterized with a proximity ligation assay. The expressions of pro-inflammatory marker interleukin 6 (IL-6) and

the generation of ROS, mitochondrial superoxide, and nitrite in neurons were assessed. Fg-induced neuronal death was assessed with a live/dead assay. We found that 1 mg/ml of Fg reduced neuronal viability (86±2% of live cells) compared to that in the control group (97±2% of live cells). A strong association of Fg with neuronal ICAM-1 and PrP^C, accompanied with the overexpression of IL-6 and the generation of ROS, mitochondrial superoxide, nitrite, and the resulting neuronal apoptosis and death were found. These effects were reduced by blocking the function of neuronal ICAM-1 and PrP^C, suggesting that the direct interaction of Fg with its neuronal receptors can induce overexpression of IL-6 and the generation of ROS, nitrite, and mitochondrial superoxide, ultimately leading to neuronal death. These effects can be a mechanism of neurodegeneration and the resultant STM reduction seen during m-mTBI and Alzheimer's disease.

Disclosures: N. Sulimai: None. **J. Brown:** None. **D. Lominadze:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: P51-OD011107
T32 MH112507

Title: Mechanisms of neuroinflammation in a monkey model of HIV associated neurocognitive disorders

Authors: *J. JEFFERSON[1,2], D. BECKMAN[1], S. R. ELIZALDI[1,3], G. B. DINIZ[1], S. OTT[1], B. A. SCHMIDT[1,3], S. S. IYER[1,3], J. H. MORRISON[1,4];
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Abstract: Despite the effectiveness of antiretroviral therapy (ART), HIV associated neurocognitive disorders (HAND) remains highly prevalent, and the aging population is at risk of developing HIV associated dementia (HAD), a severe and debilitating condition. Studies in HIV+ patients implicate acute viremia and inflammation in driving central nervous system (CNS) impairment. Non-human primates such as rhesus monkeys, are a powerful model to understand HAND pathogenesis, given the high similarities with humans in terms of the CNS structure, immune and inflammatory responses. In this pilot study, 7 female monkeys were infected with a neurotropic simian immunodeficiency virus (SIV) that induces similar pathology to that observed in HIV+ patients. An additional 4 age-matched female animals were analyzed as controls. Plasma peak viremia was observed in all animals within a few weeks, and brains were

investigated to test the hypothesis that the virus can invade and establish a viral reservoir in the CNS. Preliminary data show that, despite moderate systemic viremia (PVL range: 200-22,000 cp/ml), SIV rapidly and actively establishes a presence in the CNS, including the hippocampus and the superior temporal sulcus (STS), two major regions involved in cognitive functions. SIV replication was also observed within the cerebrospinal fluid (CSF) (23-900 cp/ml) and the virus was also detected in the choroid plexus, associated with CD4+ cells. High-resolution confocal microscopy showed blood brain barrier disruption and several sites of neuroinflammation across several brain regions. Notably, a robust neuroinflammatory response driven by reactive round-shaped microglia was observed within the hippocampus and STS. Altogether, our data shows rapid SIV establishment and dissemination within several brain regions, in a process driven by infected-activated microglia, with direct consequences for neurons and spine loss. Acute viral establishment within the CNS has significant implications for the cognitive functional deficits observed in HAND patients. An SIV-monkey model, highly translatable to humans, may help shed light on understanding the mechanisms underlying the cognitive dysfunction observed in HAND and HAD patients.

Disclosures: J. Jefferson: None. **D. Beckman:** None. **S.R. Elizaldi:** None. **G.B. Diniz:** None. **S. Ott:** None. **B.A. Schmidt:** None. **S.S. Iyer:** None. **J.H. Morrison:** None.

Poster

P209. Mechanism of Neurodegeneration I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P209.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIA R01AG069433
R01AG060718
R56063405 to GT

Title: Differential synaptic engagement by tau and Ab oligomeric species suggests distinct synaptic toxicity across Alzheimer’s disease and related tauopathies.

Authors: *M. MARCATTI, A. FRACASSI, M. MONTALBANO, N. PUANGMALAI, N. BHATT, C. NATARAJAN, B. KRISHNAN, R. KAYED, G. TAGLIALATELA;
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Abstract: Alzheimer’s Disease (AD) is characterized by gradual cognitive decline driven by the targeting of synapses by small oligomers of both Aβ (AβO) and tau (TauO), which results in synaptic dysfunction that ultimately underscores disease progression. Consequently, there is ample consensus that targeting oligomer binding to synapses would be an effective therapeutic

concept for AD. However, recent failures of clinical trials of Aβ-directed therapeutics suggest reduced effectiveness of targeting Aβ in clinically manifest AD, redirecting attention onto TauO whose levels increase later in the disease timeline. In support of this view, here we show that recombinant TauO, human brain derived tau oligomers form Alzheimer's disease (BDTO-AD) and Progressive Supranuclear Palsy (BDTO-PSP) as well as AβO, target synapses with different dynamics. Using synaptosomes isolated from the hippocampus and frontal cortex of brain mouse slices and postmortem brain specimens from cognitively intact elderly humans, we demonstrated that higher TauO concentrations effectively oucompete AβO and become the prevailing synaptic-associated species. On the other hand, although lower levels of AβO did not alter TauO binding, higher AβO concentrations resulted in increased TauO binding to human synapses. Immunofluorescence analyses of mouse primary cortical neurons treated with AβO and/or TauO fully supported these data. Consistent with these observations, functional FASS-LTP studies in human synaptosomes revealed that the TauO- induced suppression of chemical long-term potentiation (cLTP) is not affected by the concomitant presence of AβO. Moreover, depletion of synaptosome surface proteins by PK pre-treatment abolished the effect of TauO in reducing AβO synaptic binding; on the other hand, the effect of AβO in increasing TauO synaptic binding was not affected by PK. Taken together, our results provide deeper insight into the interplay between AβO and TauO that contributes to synaptic dysfunction at the basis of the cognitive decline in AD. Furthermore, we observed different synaptic engagement and internalization profiles among the analyzed BDTOs,
suggesting different synaptic toxicity of TauO across the tauopathies spectrum. Overall, our results suggest that at the advanced stages of AD, when tau levels increase, TauO become the main synaptotoxic species and likely an effective therapeutic target. Furthermore, subtle disease-specific differences in the dynamic of BDTOs engagement of synapses may underscore different clinical presentations among the tauopathy spectrum.

Disclosures: M. Marcatti: None. **A. Fracassi:** None. **M. Montalbano:** None. **N. Puangmalai:** None. **N. Bhatt:** None. **C. Natarajan:** None. **B. Krishnan:** None. **R. Kayed:** None. **G. Taglialatela:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.01

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

Support: NIH Grant R01AG023084
NIH Grant R01NS034467
NIH Grant R01AG039452
NIH Grant 5P01AG052350

NIH Grant 5P50AG005142
Alzheimer's Association strategic 509279 grant
Cure Alzheimer's Fund

Title: Analysis of blood-brain barrier permeability and cerebral blood flow in relation to APOE genotype and cognitive impairment

Authors: *G. BARISANO[1,2], A. MONTAGNE[1], D. A. NATION[7], A. P. SAGARE[3], E. JOE[4], L. M. D'ORAZIO[4], D. P. BUENNAGEL[8], M. G. HARRINGTON[8], J. M. RINGMAN[4], L. S. SCHNEIDER[5], H. C. CHUI[4], J. PA[6], M. E. LAW[9], A. W. TOGA[6], B. V. ZLOKOVIC[1];
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Abstract: Cerebral microvascular dysfunction significantly contributes to early cognitive impairment and dementia. Here we analyzed blood-brain barrier (BBB) permeability and cerebral blood flow (CBF) in relation to early cognitive decline in *APOE3*-homozygotes (ε3/ε3) and *APOE4*-carriers (ε3/ε4; ε4/ε4). A total of 143 participants (86 females [60.1%]), including 74 *APOE3*-homozygotes and 69 *APOE4*-carriers, underwent neuropsychological evaluations and magnetic resonance imaging (MRI). Dynamic contrast-enhanced and 3D pseudo-continuous arterial spin labeling (pCASL) MRI were used to estimate BBB permeability and CBF, respectively. Participants were classified in cognitively unimpaired (CU) and mild cognitive impairment according to the global Clinical Dementia Rating scale (CDR=0 and 0.5, respectively). We found that in CU and MCI individuals, there was no difference in CBF levels in grey matter (GM), hippocampus (HC), and parahippocampal gyrus (PHG) between *APOE3*-homozygotes and *APOE4*-carriers (Figure 1A-C), but higher BBB permeability values were observed in the hippocampus of *APOE4*-carriers compared with *APOE3*-homozygotes (Figure 1D). In a subgroup of 46 participants (21 CU *APOE3*-homozygotes, 18 CU *APOE4*-carriers, and 7 *APOE4*-carriers with CDR=0.5) who underwent a second MRI approximately 2 years after the first MRI, we observed a significant reduction of CBF in *APOE4*-carriers with mild cognitive impairment, but not in CU individuals (Figure 1E-F). These preliminary results suggest that BBB breakdown in *APOE4*-carriers precedes CBF alterations.

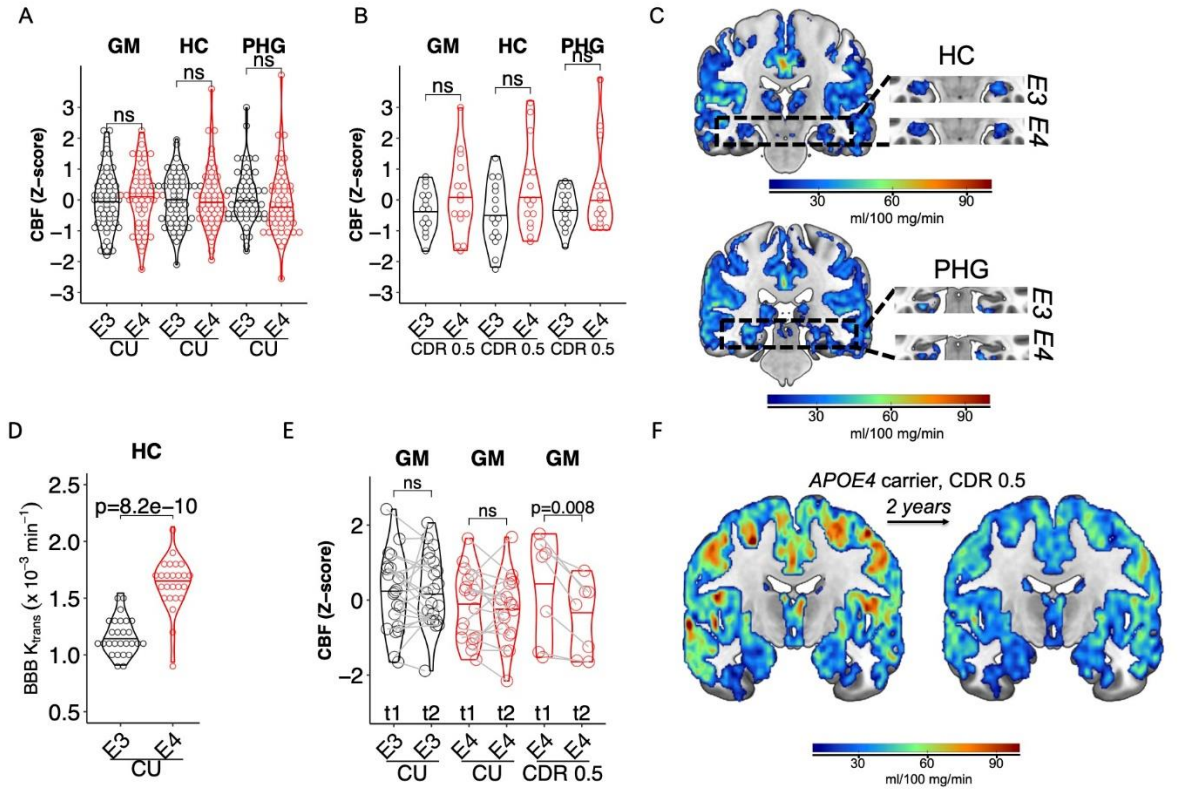


Figure 1: Standardized cerebral blood flow (CBF) levels in grey matter (GM), hippocampus (HC) and parahippocampal gyrus (PHG) in APOE4-carriers (red) compared with APOE3-homozygotes (black). No significant difference is observed in these 3 regions between APOE4-carriers and APOE3-homozygotes, both in cognitively unimpaired (CU) (**A**) and in participants with CDR=0.5 (**B**). ns, by ANCOVA controlling for age and sex. **C**) Representative CBF maps of APOE3-homozygotes and APOE4-carriers in HC (top) and PHG (bottom). **D**) BBB permeability is significantly higher in CU APOE4-carriers compared with APOE3-homozygotes. p<0.001, by ANCOVA controlling for age and sex. **E**) After 2 years follow-up, CBF in GM is significantly reduced in APOE4-carriers with mild cognitive impairment (CDR 0.5; n=7), but is not significantly altered in CU APOE3-homozygotes (n=21) and CU APOE4-carriers (n=18). Significance by paired t-test. **F**) Comparison of CBF maps in GM of an APOE4-carrier with CDR=0.5 at the first timepoint (left) and after a 2-year interval (right).

Disclosures: G. Barisano: None. **A. Montagne:** None. **D.A. Nation:** None. **A.P. Sagare:** None. **E. Joe:** None. **L.M. D'Orazio:** None. **D.P. Buennagel:** None. **M.G. Harrington:** None. **J.M. Ringman:** None. **L.S. Schneider:** None. **H.C. Chui:** None. **J. Pa:** None. **M.E. Law:** None. **A.W. Toga:** None. **B.V. Zlokovic:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.02

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

Support: NS37853
NS097805

Title: Apolipoprotein E4 impairs neurovascular regulation via brain-resident macrophages

Authors: *A. ANFRAY, Y. HATTORI, J. SEO, M. SANTISTEBAN, S. AHN, G. WANG, J. ANRATHER, L. PARK, C. IADECOLA;
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Abstract: The apolipoprotein E4 (ApoE4) causes cerebrovascular oxidative stress and impairs neurovascular regulation in humans and mice. However, the cellular sources mediating the deleterious neurovascular effect of ApoE4 remain unestablished. Perivascular and leptomeningeal macrophages (PVM) juxtaposed to pial and penetrating vessels mediate neurovascular dysfunction in models of Aβ accumulation and hypertension through oxidative stress. We tested the hypothesis that PVM are also involved in the deleterious neurovascular effect of ApoE4. We monitored the cerebral blood flow (CBF) in the somatosensory cortex of ApoE4 (human) targeted replacement (ApoE4-TR), ApoE3-TR, or wild-type (WT) mice under urethane-chloralose anesthesia by laser-Doppler flowmetry (both sexes; age 3-4 months; n=5/group). In ApoE4-TR mice, the increase in CBF produced by neural activity (whisker stimulation; WS) or by topical application of the endothelium-dependent vasodilator acetylcholine (ACh) was attenuated compared to ApoE3-TR (CBF increase-WS: ApoE3-TR: 24±4%; ApoE4-TR: 10±2%; -56%; Ach: ApoE3-TR: 20±2%; ApoE4-TR: 11±4%; -44%; $p<0.05$ from ApoE3-TR). In WT mice, neocortical superfusion of recombinant ApoE4 (rApoE4; 10 µg/ml) attenuated CBF response induced by WS (-62%; $p<0.05$ from rApoE3) or by neocortical superfusion of ACh (-48%; $p<0.05$ from rApoE3). The attenuation in the CBF increase produced by WS or ACh was associated with increased oxidative stress in PVM, assessed with DHE as a ROS marker with 2 photon microscopy *in vivo* (+33%) & FACS *ex vivo* (+38%; $p<0.05$ from rApoE3). Neocortical application of the Nox2 inhibitor gp91ds-tat (1 µM) prevented the rApoE4-induced attenuation of CBF responses to WS or ACh ($p>0.05$). Furthermore, PVM depletion using clodronate (-80%) prevented the attenuation in cerebrovascular responses and oxidative stress in WT mice induced by rApoE4 ($p>0.05$ from rApoE3). Next, we investigated if ApoE4 in PVM is required for the neurovascular dysfunction in ApoE-TR mice. To this end, after non-lethal irradiation, we transplanted ApoE4 BM into ApoE3 mice to repopulate their perivascular space with ApoE4 PVM. In ApoE3 mice transplanted with ApoE4 BM, the increase in CBF induced by WS or by ACh was attenuated compared to ApoE3 mice receiving ApoE4 BM (WS: -59%; ACh: -60%; $p<0.05$ from ApoE3◊ApoE4). These findings indicate that PVM are the critical cells required for the Nox2-derived ROS production underlying the neurovascular dysfunction induced by ApoE4 and raise the possibility that PVM may be therapeutic targets to counteract the deleterious neurovascular alterations observed in individuals with the ApoE4 genotype.

Disclosures: A. Anfray: None. **Y. Hattori:** None. **J. Seo:** None. **M. Santisteban:** None. **S. Ahn:** None. **G. Wang:** None. **J. Anrather:** None. **L. Park:** None. **C. Iadecola:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.03

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

Support: NIH Translational Science Training Grant TL1 TR002647
William and Ella Owens Medical Research Foundation
Veteran's Administration CDA2

Title: Lrp1 as a modulator of hippocampal neurogenesis and neurodegeneration

Authors: *K. DIETERT, S. MAHESULA, P. REED, S. SPRAGUE, E. KOKOVAY, N. SAYRE;
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Abstract: Neurodegenerative disease causes the death of 1/3 of senior citizens, with a prevalence projected to triple by 2050. This expected public health crisis will be exacerbated by a lack of disease-modifying treatments. As the most commonly diagnosed neurodegenerative disorder, Alzheimer's disease (AD) is characterized by a pathological loss of memory function due to hippocampal degeneration. Preservation of hippocampal neurogenesis protects from memory loss in AD, suggesting that improved understanding of molecular processes underlying neurogenesis could lead to more efficacious therapies. Neural stem cells (NSCs) migrate from the subgranular zone niche to integrate into hippocampal circuitry. The chemokine receptor CXCR4 plays a fundamental role in this migration—loss of CXCR4 activity causes hippocampal memory deficits in mouse models, and CXCR4 polymorphisms were identified as risk factors for several neurodegenerative diseases. Our lab has identified a **novel** regulator of CXCR4 - low density lipoprotein receptor related protein 1 (LRP1), which is also implicated in AD. LRP1 expression is decreased in AD patients. LRP1 is also involved in trafficking ApoE4, amyloid beta, and tau - all of which play a role in AD pathogenesis. We used a Nestin-Cre inducible mouse model to knockout LRP1 in NSCs of adult mice. We found that LRP1 knockout caused a 10-fold loss of CXCR4 expression and deficits in ischemia-stimulated migration from the subventricular zone. Male and female mice lacking NSC LRP1 also displayed spatial memory deficits at 9 months of age (6 months after knockout), suggesting dysregulated hippocampal function. Given this, **we hypothesize that LRP1 regulates CXCR4 in the subgranular zone NSCs to enhance hippocampal memory function.** Ongoing research is testing our hypothesis via hippocampal functional tests and *in vitro* trafficking/expression assays. We expect our research to elucidate a previously unknown link between three independently identified effectors of neurodegenerative disease: LRP1, CXCR4, and neurogenesis.

Disclosures: K. Dietert: None. **S. Mahesula:** None. **P. Reed:** None. **S. Sprague:** None. **E. Kokovay:** None. **N. Sayre:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.04

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

Support: College of Pharmacy and Office of Academic Clinical Affairs (FRD #19.29), University of Minnesota
NIH RF1 AG058081
NIH RF1 AG056976
NIH R21 AG056025

Title: Enhancing APOE lipidation in human iPSC-derived models with a clinically tested HDL mimetic peptide

Authors: K. FREDRIKSEN, A. VEGOE, T. O'BRIEN, *L. LI;
Univ. of Minnesota, Minneapolis, MN

Abstract: The greatest risk factor for developing late onset Alzheimer's disease (AD) is the APOE gene, which in humans has 3 alleles; APOE2 (E2) is protective against AD, APOE3 (E3) is neutral, and APOE4 (E4) increases AD risk. APOE is primarily produced and secreted by astrocytes, where it plays a key role in lipid metabolism and transport by binding lipids to form high-density lipoprotein (HDL)-like particles in the interstitial and cerebrospinal fluid. APOE interacts with amyloid-beta (Aβ), and APOE lipidation regulates Aβ aggregation and clearance. The level of lipidation is isoform dependent; E2 exhibits efficient lipidation and facilitates Aβ clearance, whereas E4 is poorly lipidated and impedes Aβ clearance. Previous studies in our laboratory have shown that a clinically tested 18-aa HDL mimetic peptide, 4F, increases APOE lipidation and secretion in primary human astrocytes as well as primary murine astrocytes and microglia. Furthermore, 4F mitigates the inhibitory effect of Aβ on APOE secretion and lipidation. Our current studies aim to corroborate these findings in human induced pluripotent stem cell (iPSC)-derived models, including astrocytes and 3D cerebral organoids. Human iPSCs from E2, E3, or E4 homozygous individuals were differentiated into cerebral organoids and treated with 4F. Preliminary results show that 4F enhances APOE secretion and lipidation in cerebral organoids derived from all three human APOE isoforms. Further studies are underway to investigate the interactions of 4F with Aβ in iPSC-derived astrocytes and the impact of 4F on AD-related pathologies in iPSC-derived cerebral organoids. The results are expected to reinforce the therapeutic potential of HDL-mimetic peptides for AD.

Disclosures: K. Fredriksen: None. **A. Vegoe:** None. **T. O'Brien:** None. **L. Li:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.05

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

Title: Corpora Amylacea Counts in the Dentate Gyrus differ by APOE genotype and degree of tau pathology in Alzheimer disease brain donors

Authors: *J. DALLMEIER[1], C. M. WANDER[4], S. MARANTO[5], V. BHAT[5], A. BARREDA[1], L. DUQUE[1], S. P. GARAMSZEGI[8], X. SUN[2], D. A. DAVIS[9], R. T. VONTELL[10], T. J. COHEN[12,6,7], W. K. SCOTT[11,1,3];
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Abstract: Introduction: Alzheimer disease (AD) is the most prevalent neurodegenerative disorder, affecting over 50 million people worldwide. Age and genetic variation can place individuals at high risk for developing AD, yet a subset of people at higher risk resist pathology and do not develop disease. Recent studies implicate corpora amylacea (CA) activity may be involved in AD pathology. Normally, CA function in the clearance of brain waste products by amassing cellular debris and extruding it into the CSF. The bundles travel to the cervical lymph nodes where they are phagocytosed. The proper functioning of CA may play a role in AD resistance, and might be influenced by age, degree of pathology, and genetic risk. AD genetic risk can be partially estimated by characterizing apolipoprotein (APOE) alleles. E4 carriers are 3-4 times more likely to develop AD than E3/E3 homozygotes, and E4/E4 homozygotes are 12-16 times as likely.
Objective: In this study, we first determined if CA are detected in the dentate gyrus and examined their frequency in a pilot study of AD hippocampal tissue samples. We then quantified differences in CA population in the dentate gyrus by APOE status, age, and extent of tau neurofibrillary tangle (NFT) pathology (B score, Montine et al., 2012).Methods: Postmortem brain hippocampal tissue sections from a pilot study of 14 AD donors were stained with hemotoxylin and eosin (H&E). Using the Motic slide scanner, sections were digitized at 40x. Aperio ImageScope software was used to manually quantify and normalize the number of CA in the dentate gyrus. A general linear model tested association of CA count in the dentate gyrus with APOE E4 carrier status (carrier vs non-carrier), adjusting for age at death, sex, and B score as covariates. Results: CA could be visualized on digital scans of H&E-stained sections. Although not statistically significant, CA count is lower in APOE E4 carriers (n = 9, mean: 3.02

CA/mm2) compared to APOE E4 non-carriers (n = 5, mean: 4.17 CA/mm2). In APOE E4 carriers higher B score (greater NFT pathology) was negatively correlated with CA count (correlation coefficient: -0.34), which was also not statistically significant.Conclusions: A small decrease in CA count was detected in the APOE E4 carriers, in whom increasing tau pathology was also correlated with lower CA counts. The correlation is intriguing and currently being tested in a larger dataset. These results suggest CA could play a role in AD pathologic progression by influencing innate tau clearance.

Disclosures: J. Dallmeier: None. **C.M. Wander:** None. **S. Maranto:** None. **V. Bhat:** None. **A. Barreda:** None. **L. Duque:** None. **S.P. Garamszegi:** None. **X. Sun:** None. **D.A. Davis:** None. **R.T. Vontell:** None. **T.J. Cohen:** None. **W.K. Scott:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.06

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

Support: NIH Grant AG062618

Title: Establishing an iPSC-derived platform to study apolipoprotein E genotype in brain cell types and its effect on extracellular vesicle cargo and bioactivity

Authors: *A. VERDUZCO[1], L. CAMPANATI[1], W. GAOR[1], K. K. BALDWIN[2], H. T. CLINE[1];
[1]Neurosci., The Scripps Res. Inst., La Jolla, CA; [2]Genet. and Develop., Columbia Univ., New York, NY

Abstract: Intercellular communication mediates astrocyte and microglia regulation of neuronal homeostasis and synaptic dynamics. The fact that in early asymptomatic Alzheimer’s disease (AD) dysregulation of astrocyte and microglia metabolism accompanies a dysregulation of synaptic function suggests an important role for intercellular communication in the onset and progression of neurodegenerative diseases like AD (Johnson et al., 2020). We are interested in studying the effects of apolipoprotein E ε4 (APOE4), a genetic risk factor for AD, on extracellular vesicles (EVs), an avenue of communication that may be implicated in regulating synaptic function. We are developing a repertoire of human induced pluripotent stem cells (iPSCs) from healthy 80+ year-old (Wellderly) donors with either homozygous APOE3 or APOE4 genotypes and are generating induced astrocytes (iAs) and microglia (iMGLs) to study cell type specific EV cargos and bioactivity. I generated iAs from APOE3 iPSCs by forced expression of transcription factors SOX9 and NFIB (Canals et al., 2018). iAs express an array of astrocytic markers by real-time quantitative polymerase chain reaction (RT-qPCR), including

S100B, GLAST, GLT-1, GFAP, HEPACAM, and AQP4, as well as S100B and GFAP by immunocytochemistry. iMGLs were generated by differentiating both APOE3 and APOE4 iPSCs first into hematopoietic progenitor cells (HPCs) and then into microglia using astrocytic and neuronal factors that mimic the environment of the developing brain (McQuade et al., 2018). At days 28 and 34 of differentiation from HPCs, iMGLs of both genotypes express macrophage lineage markers IBA1 and PU.1, microglia marker TREM2, and homeostatic microglia marker P2RY12 by RT-qPCR. By immunocytochemistry, 96% and 98% of iMGLs express IBA1, and 96% and 97% of iMGLs express TREM2 from APOE3 and APOE4 genotypes, respectively. Functional characterization revealed that more than 95% of iMGLs of both genotypes have phagocytic activity after a 16hr incubation with pre-opsonized fluorescent latex beads. Furthermore, 100% of iMGLs of both genotypes are capable of phagocytosing fluorescently labeled β-amyloid fibrils after an 18hr incubation. This repertoire of iPSC-derived astrocytes and microglia will serve as a platform on which to investigate how APOE genotype alters cell-type specific EV cargo and bioactivity.

Disclosures: A. Verduzco: None. **L. Campanati:** None. **W. Gaor:** None. **K.K. Baldwin:** None. **H.T. Cline:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.07

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

Support: NIA NRSA T32AG000255-23
NINDS R21NS105437
NINDS R37NS115439
NINDS R01NS101156

Title: Alzheimer’S disease risk factors are further dysregulated in patients with seizure history.

Authors: *A. J. BARBOUR, D. ISAACS, X. LI, D. J. IRWIN, F. E. JENSEN, D. M. TALOS; Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Alzheimer’s Disease (AD) is highly comorbid with epilepsy with more than 20% of AD patients presenting clinically identifiable seizures, which we have recently linked to an exacerbation of AD neuropathology (Gourmaud et al. 2021). Significant genetic risk factors for AD include apolipoprotein E4 (APOE4), bridging integrator 1 (BIN1), and TREM2 (triggering receptor expressed on myeloid cells 2). Interestingly, APOE4 and BIN1 have been clinically linked to epilepsy independent of AD and TREM2 has been shown to be reduced in mouse models of epilepsy. Thus, we hypothesized and that APOE4, BIN1, and TREM2 are further

dysregulated in AD patients with seizure history. To test our hypothesis, we examined temporal lobe from control and AD cases with (AD+Sz) and without (AD-Sz) known seizure history for APOE4, BIN1, and TREM2 using Luminex immunoassays and Western blot analysis. Tissue samples were obtained from the Center for Neurodegenerative Disease Research (CNDR) at the University of Pennsylvania (Philadelphia, PA) with local IRB approval. Data from all AD were compared to controls and AD+Sz were compared to AD-Sz by unpaired t-tests. Luminex multiplex assay of temporal lobe tissue revealed a significant increase in APOE4 concentrations in AD patients compared to controls (n=5-20/group, $p<0.01$) with a trend towards an increase in AD+Sz compared to AD-Sz (n=10/group, $p=0.06$). Further, TREM2 concentrations were significantly reduced in AD cases compared to control (n=5-20, $p<0.05$), and in AD+Sz compared to AD-Sz (n=10, $p<0.05$). Western blot analysis of temporal lobe revealed no difference in BIN1 expression between AD and controls, but a significant decrease of BIN1 levels in AD+Sz versus AD-Sz (n=8-9, $p<0.01$). Overall, our data demonstrate that AD risk factors are further dysregulated in patients with seizure history and may underlie seizure exacerbation of AD neuropathology.

Disclosures: A.J. Barbour: None. **D. Isaacs:** None. **X. Li:** None. **D.J. Irwin:** None. **F.E. Jensen:** None. **D.M. Talos:** None.

Poster

P227. Apoe and Associated Pathways II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P227.08

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

Support: USC ADRC Developmental Pilot Award (NIH P30AG066530)
CZI Imaging Scientist Award (2020-225670)

Title: Machine learning classification and quantification of Alzheimer’s disease pathology within human hippocampal subregions

Authors: L. KOROBKOVA[1], K. T. JONES[2], R. P. CABEEN[1], C. A. MILLER[2], *M. S. BIENKOWSKI[1];
[1]USC Stevens Neuroimaging and Informatics Inst., Keck Sch. of Med. of USC, Los Angeles, CA; [2]Dept. of Pathology, USC, Los Angeles, CA

Abstract: Alzheimer’s disease (AD) has recently become more appreciated as a neurodegenerative disease with multiple subtypes and pathological heterogeneity. AD pathology (amyloid plaques and tau neurofibrillary tangles (NFTs)) is differentially distributed within anatomical structures over progressive disease stages, but quantification of pathology across multiple brain subregions within whole post-mortem tissue sections is difficult and is most often

qualitatively scored by a trained neuropathologist. To rapidly quantify the distribution of AD pathology, we developed a machine learning and image registration approach to classify various pathological features from histologically-stained hippocampal/temporal lobe tissue sections obtained from the USC ADRC Neuropathology Core. For each patient, two adjacent coronal sections stained for amyloid (4G8 immunostaining) and tau (Gallyas histochemical stain) were imaged using a whole slide scanning microscope. Tissue sections were manually annotated to create subregion segmentation masks used to delineate anatomical subregion boundaries. To differentially characterize anatomical features of AD pathology, we classified and segmented different types of histology labeling: amyloid (4G8 intracellular vs. extracellular dense vs. extracellular diffuse), tau (intracellular Gallyas), and neuritic plaques (extracellular colocalization of 4G8 and Gallyas labeling). Our cross-sectional study reveals specific alterations of neuritic plaques and NFTs with respect to APOE gene variants and AD progression. As expected, the number of dense neuritic plaques and tau NFTs increased with AD progression. However, we also found anatomical subregion-specific effects of APOE genotype on tau and amyloid pathology. Additionally, we found that diffuse amyloid labeling increased with AD progression significantly greater than dense neuritic plaque formation and was mostly concentrated in entorhinal cortex, parasubiculum, and presubiculum regions. Overall, the precise anatomical localization of pathological aggregates is consistent with a model of a disease progression along specific axonal pathways.

Disclosures: L. Korobkova: None. **K.T. Jones:** None. **R.P. Cabeen:** None. **C.A. Miller:** None. **M.S. Bienkowski:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.01

Topic: D.03. Somatosensation – Pain

Support: NS103350-02S1
W81XWH1810627

Title: Cgrp signaling contributes to mild traumatic injury-induced post-traumatic headache and cognitive deficit

Authors: *K. CZERPANIAK[1], Y.-Q. CAO[2];
[2]Anesthesiol., [1]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Post-traumatic headache (PTH) and memory loss are some of the most common symptoms following mild traumatic brain injury (mTBI). Despite the prevalence of mTBI in humans, the underlying mechanisms remain unclear and treatment options are unavailable.

Previous research suggests a role for calcitonin gene-related peptide (CGRP) signaling in PTH, which raises the possibility of treating mTBI with therapeutics targeting CGRP. Other studies reported that early and repeated anti-CGRP monoclonal antibody treatment blocks behaviors related to PTH, but whether CGRP signaling contributes to mTBI-induced cognitive impairment has not been tested. In this current study, we used both genetic and pharmacological approaches to test whether CGRP signaling plays a role in PTH-related behaviors and cognitive deficits in a mouse model of mTBI. First, mice were subjected to an mTBI procedure that resulted from a closed head weight-drop. Then, PTH was evaluated by measuring facial mechanical thresholds using von Frey filaments. Recognition and spatial memory were evaluated with the novel object recognition (NOR) test and object location test (OLT). Both wild-type male and female mice subjected to mTBI showed profound reduction of facial mechanical threshold as well as deficit in NOR and OLT assays. When compared to their wild-type controls, neither male nor female CGRP knockout mice exhibited increased sensitivity to von Frey filaments after mTBI, supporting that endogenous CGRP signaling mediates mTBI-induced PTH. Similar to these results, CD-1 mice that received anti-CGRP monoclonal antibody treatment immediately after injury and then every six days until day 18 post-mTBI did not develop PTH-related behaviors when compared to the mTBI group that received control IgG treatment. However, 3-14 days after the cessation of anti-CGRP antibody treatment, 60% of these mice showed a significant reduction in facial mechanical threshold, suggesting that long-term antibody treatment may induce compensatory neurochemical changes that affect basal nociception. Interestingly, results from the NOR test reveal that both male and female CGRP knockout mice did not display any deficit after mTBI, but did show poor performance in the OLT. Similarly, the anti-CGRP monoclonal antibody completely prevented mTBI-induced NOR deficit, but these mice still exhibited a spatial memory impairment in the OLT. In conclusion, these results suggest that CGRP signaling plays a role not only in mTBI-induced PTH, but in non-spatial recognition memory decline as well.

Disclosures: K. Czerpaniak: None. **Y. Cao:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.02

Topic: C.10. Brain Injury and Trauma

Support: VA Grant 1BX004256
VA Grant 1RX001141
VA Grant IK6BX005235
NIH Grant T32AI132164

Title: Complement mediated cognitive decline in chronic phases post traumatic brain injury

Authors: *K. MALLAH[1], C. COUCH[2], M. ALSHAREEF[3], D. BORUCKI[4], X. YANG[1], A. ALAWIEH[5], S. TOMLINSON[1];
[1]Microbiology and Immunol., [2]Hlth. Sci. and Res., [3]Neurolog. Surgery, [4]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [5]Neurosurg., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Traumatic Brain Injury (TBI) can result in dementia and cognitive decline in chronic phases after injury. Studies have shown a significant increase in the hazard ratio of developing dementia after sustaining a brain injury, a ratio which lasts for years after initial insult. The underlying pathological mechanisms that link TBI to cognitive decline are not well understood. We have previously shown that the complement system is implicated in mediating secondary injury and cognitive deficits up to 1 month after murine TBI. In this work, we studied the role of complement in mediating ongoing cognitive decline measured at 3 and 6 months after murine controlled cortical impact. We employed a battery of behavioral tasks to assess cognitive performance after TBI, and results were correlated with histopathological analysis focusing on complement deposition and glial response (microglia and astrocytes). We also assessed the therapeutic potential of complement inhibition for reducing a chronic neuroinflammatory response and reversing cognitive decline. Cognitive decline after TBI in untreated animals was coupled with complement deposition in the perilesional area, as well as with expanded microgliosis and astrogliosis to the contralateral hemisphere of brains by 3 months after TBI. However, cognitive decline, complement deposition and gliosis, measured at 3 and 6 months after TBI, was reversed by treatment with the complement inhibitor CR2-Crry, even when treatment was initiated 2 months after insult. Lesion volume was also reduced in CR2-Crry vs. vehicle treated mice at 6 months post-TBI, although not at 3 months post-TBI. Using high resolution fluorescence microscopy, we linked cognitive decline to a reduction in synaptic density which resulted from microglial phagocytosis of complement (C3) opsonized synapses, and which was reversed with complement inhibition. For CR2-Crry treatment starting at 2 months after injury, a weekly dose of CR2-Crry was required for improvement, and a short transient treatment paradigm was not protective at 6 months after injury. Taken altogether, the data show persistent complement deposition in the brain at chronic timepoints after initial insult, and which is correlated with ongoing cognitive decline and a persistent neuroinflammatory response, all of which can be reversed by complement inhibition.

Disclosures: K. Mallah: None. **C. Couch:** None. **M. Alshareef:** None. **D. Borucki:** None. **X. Yang:** None. **A. Alawieh:** None. **S. Tomlinson:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.03

Topic: C.10. Brain Injury and Trauma

Support: P20 GM109089/GM/NIGMS NIH HHS/United States

Title: The role of spreading depolarizations in the acute behavioral deficits associated with mild traumatic brain injury in mice

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Abstract: It is estimated that 42 million people experience a mild traumatic brain injury (mTBI) worldwide every year. mTBIs are often diagnosed by the acute symptoms including nausea, dizziness, headaches, and disorientation. The underlying cellular and physiological mechanisms of the acute symptoms are currently unknown. It has long been hypothesized and recently confirmed that spreading depolarizations (SDs) occur in mTBIs. Spreading depolarizations are massive waves of complete depolarization and the loss of chemical gradients that travel slowly through grey matter resulting in the suppression of cortical activity for multiple minutes. We hypothesize that SDs are a key underlying mechanism of the acute behavioral deficits associated with an mTBI. We will utilize a closed skull impact model in mice that reproducibly initiates an SD. We assess acute behavior in an open field arena with motion tracking software and a series of behavioral tasks that are collectively scored as the neurological severity score (NSS). To investigate the role SDs have in the acute behavioral deficits, we use KCl and optogenetic approaches to initiate an SD in the absence of an impact. We found that mTBIs are associated with a significant increase ($p= 0.0042$) in the NSS relative to sham treated animals ($n=8$). Interestingly, in a subset of mTBI animals there was no SDs initiated by the impact ($n=2$). These animals did not display deficits in the NSS relative to sham treated animals. SDs alone (initiated via KCl or optogenetics) were associated with similar deficits in the NSS as the mTBI animals but were significantly different from their respective saline or sham controls. Current studies are using pharmacological approaches (memantine) to block the SD in the mTBI animals and investigating the acute behavior. Collectively, these studies suggest that SDs play a key mechanistic role in the acute behavioral deficits associated with mTBIs. Understanding the cellular and physiological mechanisms underlying the acute behavioral deficits associated with mTBIs will identify particular targets for intervention and promote brain recovery and repair.

Disclosures: N.J. Pinkowski: None. **M. Martinez:** None. **C.J. Mehos:** None. **R.A. Morton:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.04

Topic: C.10. Brain Injury and Trauma

Support: CSF Grant UIP-2017-05-9517

Title: Chemically-functionalized single-walled carbon nanotubes increase the expression of glial fibrillary acidic protein in the mouse primary astrocytes exposed to severe in vitro traumatic brain injury

Authors: *K. PILIPOVIć[1], I. JURIčKI[3], T. MLADENIć[2], A. HAREJ HRKAć[1], N. GRŽETA[1], V. PARPURA[4], G. ŽUPAN[1];
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Abstract: Astrocytes are central nervous system (CNS) cells with an important role in neuronal regeneration following brain injury. This study aimed to investigate the effects of single-walled carbon nanotubes (SWCNTs), chemically functionalized with poly-m-aminobenzene sulfonic acids (PABS), on the oxidative protein damage and the expression of the glial fibrillary acidic protein (GFAP) and the inducible nitric oxide synthase (iNOS) in astrocytes exposed to an *in vitro* model of severe traumatic brain injury (TBI). Primary mouse astrocytes were grown on 6-well plates with deformable membranes and subjected to stretch injury, mimicking severe TBI. PABS-SWCNTs were applied to severely injured cells at 1 h post-injury. Cells were collected at 24 h following stretch and the lysates were prepared for the analyses. The dot blot method was used to detect the levels of oxidatively damaged protein. Western blot analyses were used to determine the expressions of GFAP and iNOS. Non-treated cells were used as the control. Dot blot assay demonstrated that the stretch injury and the treatment with PABS-SWCNTs did not cause a significant change in the levels of oxidatively damaged proteins. We observed no change in the expression of the inflammatory marker iNOS. Conversely, the application of PABS-SWCNTs significantly elevated the expression level of GFAP compared to the results obtained in the samples of both control cells and the non-treated, severely stretched astrocytes. Our preliminary results suggest that the application of the investigated nanomaterial, applied as a colloidal solute, to the primary astrocytes exposed to severe *in vitro* TBI causes an increase in the GFAP levels. This is a finding with possible relevance related to the role of GFAP in the trafficking of glutamate transporters which needs to be further explored. This research was fully supported by the Croatian Science Foundation grant UIP-2017-05-9517 to KP.

Disclosures: K. Pilipović: None. **I. Jurički:** None. **T. Mladenić:** None. **A. Harej Hrkać:** None. **N. Gržeta:** None. **V. Parpura:** None. **G. Župan:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.05

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant T32NS077888
NIH Grant T32GM007250

Title: Role of mitochondrial fission in progressive neurodegeneration and memory deficit after traumatic brain injury

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Abstract: In the past decade, there has been an increasing incidence of traumatic brain injury (TBI), which is linked to a 2-4-fold increase in risk for developing dementia later in life. We are modeling this in our laboratory using an injury system that induces components of global concussion, acceleration/deceleration, and blast wave exposure. TBI in people presents as a progressive and chronic neurodegenerative injury characterized by neuropsychiatric impairment, including cognitive deficits and depression. Currently, there are no available treatments that slow the progression of neurodegeneration and associated symptoms after TBI. Mitochondrial fission-fusion dynamics have been identified as an important component of pathogenesis in Alzheimer's disease, which TBI patients are at an especially elevated risk of developing. In the present study, we investigate the potential efficacy of a pharmacologic inhibitor of mitochondrial fission, called P110, in mitigating the progression of symptoms after TBI.
Male and female C57/bl6 mice were subjected to either TBI or sham injury at 2 months of age, and subsequently treated with either P110, a specific peptide inhibitor of mitochondrial fission, or vehicle control by daily intraperitoneal injection. Following treatment, mice were tested for neurocognitive deficits using a battery of behavioral tests, including novel object recognition (NOR), open field test, elevated plus maze, and tail suspension test. Brain tissues were then processed for western blot analysis and staining.
Drp1, the primary mediator of mitochondrial fission, is the target of the P110 peptide inhibitor. Mice subjected to TBI showed an elevation in Drp1 expression 24 hours post-injury, with levels returning to normal after 2 weeks. This suggests a transient elevation in mitochondrial fission. Two weeks after TBI, mice showed a memory deficit in NOR, and P110 treatment was protective. Treatment was then ceased, and the same mice, when tested in NOR 6- and 9- months post-injury, showed sustained memory protection. When P110 treatment initiation was delayed until 8 months after injury, it did not restore normal cognition. All behavioral experiments were blinded for analysis and repeated in at least 2 separate cohorts. No sex differences were observed.
Our results indicate that early inhibition of mitochondrial fission after TBI is protective against neurodegeneration and memory loss, and that this brief treatment produces a lasting protective effect from chronic memory deficits as well. Future directions will focus on investigation of the

mechanisms by which imbalance of mitochondrial fission and fusion after TBI mediates neurodegeneration.

Disclosures: P.S. Sridharan: None. **Y. Koh:** None. **E. Miller:** None. **E. Vázquez-Rosa:** None. **M. Shin:** None. **K.Z. Franke:** None. **X. Qi:** None. **A.A. Pieper:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.06

Topic: C.10. Brain Injury and Trauma

Support: Rowan University Osteopathic Heritage Foundation to DMD
Rowan University School of Osteopathic Medicine to BDW

Title: Deficits in catecholamine signaling of the prefrontal cortex following repetitive mild traumatic brain injury

Authors: L. HORVAT[1], A. FOSCHINI[2], D. P. FOX[2], J. P. GRINIAS[1], B. D. WATERHOUSE[2], ***D. M. DEVILBISS**[2];
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Abstract: Recurring mild traumatic brain injuries (TBI) are a large class of the 1.7-3.8 million reported injuries each year. Regardless of severity, TBI is a complex pathophysiological process resulting in behavioral and cognitive deficits that include impairments in arousal, attention, decision-making, and other executive functions. Repetitive mild injuries can produce cumulative and long-term cognitive deficits and increase the susceptibility for further injury after each mild TBI. Catecholamine systems, including the locus coeruleus (LC)-norepinephrine (NE) and the ventral tegmental-dopamine (DA) pathway, are critical regulators of these higher executive processes affected by TBI. Furthermore, impaired NE and DA signaling within the prefrontal cortex (PFC) circuitry is implicated in the pathogenesis of the cognitive and neuropsychiatric symptoms following *single* moderate to severe TBI. However, the effects of mild TBI and *repetitive* TBI on catecholamine system function are poorly understood. Methylphenidate (MPH) and other psychostimulants that elevate NE and DA within the PFC have been used as a putative treatment strategy to recover normal cognitive function after injury. However, limited and inconsistent findings exist on the efficacy of MPH for treating the cognitive impairments following mild TBI due to small sample sizes, poor patient stratification, and limited dose ranges. The objectives of the current study were to determine the extent of NE and DA dysfunction and response to therapeutic doses of MPH in rats following a repetitive mild closed-skull TBI. Baseline NE efflux within the PFC and responsiveness to MPH challenge were

significantly impaired after repetitive mild TBI. However, simultaneous quantification of DA within the PFC did not show comparable deficits in baseline levels or responsiveness to MPH challenge. Behavioral measures of arousal demonstrated reduced wakefulness only after repeated mild TBI. Finally, important sex differences were found, with males exhibiting substantially lower baseline NE levels and responses to MPH challenges compared to females after repeated mild TBI. These findings provide critical insight into the sensitivity of catecholamine systems to repeated mild TBI and support for the role of noradrenergic fiber injury in the cognitive deficits resulting from repeated injury.

Disclosures: L. Horvat: None. **A. Foschini:** None. **D.P. Fox:** None. **J.P. Grinias:** None. **B.D. Waterhouse:** None. **D.M. Devilbiss:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.07

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # 1 I01 RX003123-01A1 (B3123-1), from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D).

Title: Innovative treatment for TBI-microbleed induced chronic inflammation in neural substrates for motor, cognitive and anxiety disorders

Authors: *P. BOSE[1,2,3], J. HOU[1,2], S. TSUDA[2,1], D. PLANT[1], B. RENGERT[1], R. RUPARELIYA[1], K. ZHANG[1], H. SHROCK[1], G. M. DOOLEY[1], S. LULU[1], A. CHAMBERS[1], C. BLUM[1], R. J. BERGERON[5], F. J. THOMPSON[1,4];
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Abstract: Acceleration/deceleration closed head traumatic brain injury (CH-TBI) induces damage of micro-vessels which results in endothelial shear injury, blood brain barrier (BBB) dysfunction, and micro-bleeding. Microbleed-derived iron can provide enduring oxidative stress and inflammation, further breakdown of the BBB tight junctions, and cell death through multiple inflammatory pathways. The current studies tested the influence of CH-TBI (n=20) and the therapeutic impact of an iron chelator (n=19) on hallmark chronic disabilities in a rodent model. Mild/moderate CH-TBI was produced using our previously reported protocol (450 g/1.25m). An iron chelator, NaHBED (n=10) treatment was initiated at PO Day-0 and continued for 2 weeks (50 mg/kg/day, SQ). The control animals received an equal volume of saline (SQ; n=7). Tests for

motor, anxiety, and cognitive functions were conducted at multiple time points during 9 months of post-injury study. 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 to date revealed long-term enduring disabilities in motor/vestibulomotor, anxiety, and cognitive behaviors following CH-TBI, and significant reductions in these disabilities in the NaHBED treated animals. IHC and histology studies of TBI tissues showed patterns of a) iron deposition and disruption of BBB, b) increased expression of markers for inflammation (activation of NF-kB via TLR4 pathway, proinflammatory cytokines, proIL-1β, TNFα, etc.), c) pyroptosis of neuronal cells in specific regions essential for the studied motor, cognitive, and anxiety behaviors, and d) loss of regulatory noradrenergic and trophic supports in these regions. Tissue from the NaHBED-treated animals exhibited robust normalization of each of these markers. Collectively, our studies demonstrate that: 1) TBI-induced microbleeds contribute to the development and persistence of multiple chronic disabilities, 2) iron deposited via TBI induces BBB disruption, and accelerates neuroinflammation and neuronal cell death, and 3) this collective trauma portfolio of chronic disability and inflammation can be attenuated by an iron chelator therapy. Taken together, iron chelator treatment offers the potential for a mechanism-based therapy that addresses a significant contributor of long-term TBI disabilities, contributes to trophic support for neuronal and vascular healing, and enhances neuroplasticity for adaptive compensation.

Disclosures: P. Bose: None. **J. Hou:** None. **S. Tsuda:** None. **D. Plant:** None. **B. Rengert:** None. **R. Rupareliya:** None. **K. Zhang:** None. **H. Shrock:** None. **G.M. Dooley:** None. **S. Lulu:** None. **A. Chambers:** None. **C. Blum:** None. **R.J. Bergeron:** None. **F.J. Thompson:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.08

Topic: C.10. Brain Injury and Trauma

Title: Neuroprotective Effects of Tamoxifen in Xenopus laevis Model of Focal Impact Injury

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Abstract: Traumatic brain injury (TBI) is the leading cause of disability worldwide and has produced lasting effects on the lives of over 5.3 million people in the U.S. alone. While the initial injury causes mechanical damage, the secondary injury produced by the inflammation cascade around the site of a lesion can be equally harmful, producing lasting motor, sensory, behavioral, and cognitive deficits. The complex role of astrocytes in mounting and modulating the inflammatory cascade after an injury has been extensively studied, as have the

neuroprotective effects of estrogen compounds in mammalian model organisms. Although astroglial cells are essential in limiting neuronal damage, regulating inflammation, and repairing tissues following a TBI, estrogen compounds that reduce the hyperactivation of astrocytes have been shown to reduce secondary injury without sacrificing the necessary post-injury activity of astrocytes.
Objective: To evaluate the effect of tamoxifen treatment on neuronal survival and astrocyte activation near the wound site, edema accumulation levels, and behavioral output after an induced focal impact brain injury in *Xenopus laevis*.
Design: In this study, we induced a focal impact (FI) injury to the optic tectum in the midbrain of stage 55 *Xenopus laevis* tadpoles. Animals were randomly divided into sham injured (n=18, FI injured + vehicle treated (n=18), FI injured + tamoxifen treated (n=18), and sham + tamoxifen (n=18), in accordance with the performed power analysis. Behavioral assays were performed for consecutive days following treatment and/or injury. At time points of 3, 24-, 48-, 72-, and 168-hours post-injury, brains were collected, and immunofluorescence staining, edema quantification, and qRT-PCR were performed.
Results: Our results demonstrate that administering tamoxifen immediately after a focal impact injury produces sustained upregulation of critical neuroprotective genes: *timp 1, bdnf, manf, and steap4*. It also appears to modulate the injury-driven increase in brain water content and reduce the elevated expression of *aquaporin-4,* suggesting a possible role in regulating edema. Tamoxifen also attenuated behavioral deficits of injured animals in visual preference, open field, and spot avoidance assays. These results suggest that tamoxifen is neuroprotective: it improves behavioral recovery, modulates neuronal death and astrocyte activation, and decreases edema accumulation. These findings, consistent with published literature in mammalian models of spinal cord injury, suggest that tamoxifen could represent a potential therapeutic intervention in regulating inflammation following a focal impact injury.

Disclosures: S. Spruiell Eldridge: None. **A.K. Sater:** None.

Poster

P348. Traumatic Brain Injury: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P348.09

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # 1 I01 RX003123-01A1 (B3123-1), from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D).
Center Award # B9252C from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Therapeutic impact of noradrenergic upregulation following closed head traumatic brain injury (CH-TBI)

Authors: *F. J. THOMPSON[1,2], J. HOU[1,3], S. TSUDA[1,3], D. PLANT[1], S. NADEAU[1,4], P. BOSE[1,3,4];
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Abstract: TBI induces significant and enduring cognitive, anxiety, and motor disabilities. Our previous and current studies demonstrate significant injury of the locus coeruleus nucleus, projection fibers, and systematic loss of noradrenergic (NA) innervation to cognitive, anxiety, and motor neural substrates following acceleration/deceleration CH-TBI. Behavioral/physiological function studies have also revealed that these losses of NA innervations correlated with alterations in cognitive, anxiety, and motor functions. NA projections are critical for regulation of the excitability of spinal- and vestibulo-spinal reflexes, motor cortex gait coordination, blood brain barrier (BBB), inflammatory signaling, and BDNF production. Our preliminary studies have also revealed chronic inflammation in the essential neural substrates for cognitive, anxiety, and motor functions. Accordingly, use of agents that target and upregulate NA expression, correlated with measures of TBI-induced disabilities, could provide additional insights into the role of NA injury in TBI pathobiology. Thus, the objective of this study was to test the therapeutic impact of NA agonists on hallmark TBI disabilities in a rodent model of CH-TBI. We hypothesized that a desirable strategy for NA therapy following TBI would be intrinsic upregulation of NA expression to produce a more integrated and regulated NA function in downstream innervated substrates. We compared behavioral/physiological function measures in TBI and TBI/NA agonists treated animals to evaluate the potential therapeutic impact of NA upregulation on TBI disabilities. We used Methylphenidate, which blocks the norepinephrine (NE) transporter, prolonging the re-uptake of NE, and upregulates the effectiveness of intrinsically distributed NE. In addition, we tested therapy with phenylephrine and ephedrine (direct adrenergic agonists) to evaluate the more selective contribution of adrenergic alpha-1 vs alpha & beta receptor activation, respectively, for treatment efficacy. These studies were carried out in CH-TBI and CH-TBI/treated animals. Mild/moderate TBIs were produced by a 450-gram weight drop (1.25 meter) impact on the helmeted head. Methylphenidate (0.5 mg/kg; oral), phenylephrine (0.6 mg/kg; SQ), and ephedrine (3.8 mg/kg; SQ) treatments were initiated one week following injury. Although differential, collectively, significant decreases were observed in the TBI-induced cognitive, anxiety, and motor disabilities in the treated animals compared to saline-treated TBI animals. However, the therapeutic impact of methylphenidate was most robust and consistent across all three behavioral measures

Disclosures: F.J. Thompson: None. **J. Hou:** None. **S. Tsuda:** None. **D. Plant:** None. **S. Nadeau:** None. **P. Bose:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 1P50DA044121-01A1
NSF Grant DGE-1324585

Title: Local and global blood flow and arrival time (ASL) associations with pain intensity in patients with chronic back pain

Authors: *L. HUANG[1], A. VIGOTSKY[2], Y. CHEN[1], T. PARRISH[1], T. J. SCHNITZER[1], A. V. APKARIAN[1];
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Abstract: Arterial spin labeling (ASL) is a noninvasive magnetic resonance imaging (MRI) technique that uses magnetically labels arterial blood to obtain estimates of cerebral blood flow (CBF) and bolus arrival time (BAT). Yet, to date, its application to the study of the patients with chronic back pain (CBP) has been relatively limited. A total of 86 CBPs (male = 46; age = 56 ± 13 years old; BMI = 30 ± 6; pain (numeric rating scale, NRS) = 69/100 ± 15/100; pain duration = 45 - 2661, median = 417 weeks) were included in this study and scanned on a clinical 3 Tesla Siemens Magnetom Prisma scanner. CBF and BAT images were registered to MNI space and mean values of both CBF and BAT images within gray matter (GM) were calculated, referred as $mCBF_{GM}$ and $mBAT_{GM}$. Multiple regression revealed that NRS, together with age, gender, BMI, and log(pain duration) accounted for 16.50% of variance of $mCBF_{GM}$ ($F(5, 76) = 3.08$, $p = 0.016$) and 21.98% of variance of $mBAT_{GM}$ ($F(5, 76) = 4.28$, $p = 0.002$), where NRS was significantly associated with $mCBF_{GM}$ ($p = 0.026$) and $mBAT_{GM}$ ($p = 0.015$), respectively. To identify brain regions in which NRS was associated with CBF or BAT, the participants were divided into discovery ($n = 50$) and validation ($n = 36$) groups matched on age, gender, BMI, and log(pain duration). One cluster for CBF (left temporal pole, $p < 0.001$ (discovery) and $p = 0.02$ (validation)) and 2 clusters for BAT (left temporal pole, $p < 0.001$ (discovery) and $p = 0.03$ (validation); right superior parietal lobule, $p = 0.002$ (discovery) and $p = 0.03$ (validation)) were revealed (discovery) and replicated (validation) after family-wise cluster error correction ($p < 0.01$) was applied. The finding that ASL signal was both globally and locally associated with pain intensity after adjusting for the effects of age, gender, BMI, and pain duration suggests it may provide additional information to increase our understanding of the neuropathology in CBP patients. However, the relationship between ASL signal and other, more established fMRI techniques, such as functional connectivity and BOLD variability, remain to be elucidated.

Disclosures: L. Huang: None. **A. Vigotsky:** None. **Y. Chen:** None. **T. Parrish:** None. **T.J. Schnitzer:** None. **A.V. Apkarian:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01NS118563
NIH K01AR071504
Rita Allen Foundation
Bertha Rosenstadt Endowment Fund

Title: Hippocampal volume, FKBP5 genetic vulnerability and childhood trauma compound risk for chronic multisite pain in the UK Biobank cohort

Authors: *L. J. AYOUB[1,2], J. J. LOBO[3], S. LINNSTAEDT[3], M. MOAYEDI[1];
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Abstract: Chronic musculoskeletal pain affects up to 47% of individuals around the world. Chronic multisite musculoskeletal pain (CMP) affects multiple body sites, and its severity is characterized by the number of body sites affected for more than three months. However, risk factors contributing to the development and severity of CMP remain unknown. Risk factors for chronic musculoskeletal pain include a smaller right hippocampus, a risk allele of the *FKBP5rs3800373* and early childhood trauma. However, whether the relationship of these factors compound the effect of CMP severity has not been investigated. Here, we test the relationship between these factors using a large cohort of individuals with chronic musculoskeletal pain from the UK Biobank. We tested three preregistered hypotheses: (1) *FKBP5rs3800373,* and (2) hippocampal volume, and (3) their interaction is associated with CMP. In secondary analyses, we assessed whether this interaction is moderated by early childhood trauma. We used linear regression modelling to test these hypotheses. We included 36,822 participants (52.4% women; 63.6 ± 7.5 years). First, we did not find statistically significant main effect associations between *FKBP5rs3800373* (β=-0.003, p=0.701) and CMP nor hippocampal volumes (left: β=-0.005, p=0.238; right: β=-0.007, p=0.130) and CMP. However, we did observe a statistically significant interaction between *FKBP5rs3800373* and right hippocampal volume (β=-0.020, p_{corr}=0.01), but not left hippocampal volume (β=-0.015, p_{corr}=0.12) on CMP. Specifically, individuals with two *FKBP5rs3800373* risk alleles had the strongest association between right hippocampal volume and CMP, such that smaller right hippocampus was statistically associated with increased CMP severity (β=-0.031, p=0.045). In a sub-cohort of individuals with available data on early childhood trauma (n=25,280), the interaction between childhood trauma severity (frequency of childhood trauma types) and right hippocampal volume on CMP was significant in individuals with 1 (β=-0.084, p=0.006) or 2 (β=-0.168, p=0.042) *FKBP5rs3800373* risk alleles. This suggest that in individuals with increased levels of childhood trauma and with *FKBP5rs3800373,* the smaller right hippocampus was associated with CMP severity. Overall, our findings show that the interaction between a

smaller right hippocampus and *FKBP5rs3800373* genetic risk is associated with CMP, and this association is further moderated by childhood trauma. Our results identify risk factors for CMP and indicate that further work studying causal relationships between these factors is warranted.

Disclosures: L.J. Ayoub: None. **J.J. Lobo:** None. **S. Linnstaedt:** None. **M. Moayedi:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.03

Topic: D.03. Somatosensation – Pain

Support: NIDA R01 DA046064
NIMH 3R01 MH076136
NIBIB R01 EB026549

Title: Resting state fMRI hyperalignment reveals a generalizable fine scale structure of evoked cortical response to noxious stimuli

Authors: *B. PETRE[1], M. CEKO[2], N. P. FRIEDMAN[3], M. C. KELLER[3], M. A. LINDQUIST[4], T. D. WAGER[1];
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Abstract: Cortical feature representations include meso-scale information that is heterogeneous across individuals. This has limited their characterization beyond individual brain structures where simple functions like columnar stimulus mappings can be studied in individual subjects. Connectivity hyperalignment provides an indirect but scalable and generalizable alternative: matching functional connectivity profiles across subjects to build a common model of brain function that aligns fine scale idiosyncratic task evoked responses. We used resting state fMRI to hyperalign responses to noxious heat and pressure stimulation of the fingers (N=104 subjects), stimuli that are likely distinguished at the mesoscale. We found hyperalignment reduced interindividual differences in evoked responses and improved local nociceptive modality discrimination throughout the cortex.
Hyperalignment revealed widely distributed fine-grained, striated patterns of evoked responses. This coincided with significant improvements in searchlight-based between-subject correlations (BSCs) of evoked responses for 88% of the cortex and decreases in BSCs for 0.06% of cortex relative to non-hyperaligned data (permutation test, FDR $p < 0.05$). The largest improvements were in the insula and premotor areas, including putative primary nociceptive areas like SII. We performed a searchlight sweep of each hemisphere with linear support vector machines to test

out-of-subject discrimination of noxious modality. Without hyperalignment, 31.9% of the cortex discriminated modality (>55% accuracy, FDR $p<0.05$, max 71%, contralateral PFm) vs. 73.2% of the cortex with hyperalignment (max 75%, contralateral SII). No searchlight showed significant decrease in accuracy after hyperalignment. Improvements were predominantly outside of typical thermosensory areas, especially in dorsolateral prefrontal cortex and posterior parietal cortex (peak improvement: area 8Av, proportion z-test: $z=6.3$, classification improvement = 0.18, $p < 1e-5$), but significant improvements were also present in somatomotor cortices (best contralateral improvement: BA1, $z=5.6$, 0.15 accuracy improvement, $p = 0.0002$). We show connectivity hyperalignment effectively captures a common model of cortical nociceptive representation. This model reveals that different modalities of noxious stimuli have divergent effects on cortical activity that are fine scaled but widespread, distributed outside of classic thermosensory areas, and translate across individuals. This demonstrates the viability of hyperalignment for characterizing a generalizable fine structure of cortical nociceptive processing.

Disclosures: B. Petre: None. **M. Ceko:** None. **N.P. Friedman:** None. **M.C. Keller:** None. **M.A. Lindquist:** None. **T.D. Wager:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.04

Topic: D.03. Somatosensation – Pain

Support: Hitchcock Foundation Pilot Grant
NIH Grant R01 MH076136

Title: Deep-phenotyping of real and imagined pain across eight-body sites in an n-of-1 design

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Abstract: The neural representation of pain reflects some combination of the processing of incoming sensory and contextual information that may be organized into separable components of neural activity pertaining to location, stimulation, and aversiveness. We had a single-subject (the first author, a 32 year-old Asian American male) undergo repetitive functional magnetic resonance imaging (fMRI) scans to deeply phenotype the neural activity of each pain component. To develop these components, eight body-sites (left and right cheek, left and right forearm, left and right calf, chest, and abdomen) were thermally stimulated with a Peltier thermode at painful heat and non-painful warm intensities repetitively for 12-13 seconds for 34 runs over seven sessions (days). Concomitantly, the subject was periodically asked to imagine each body site

being intensely burned by the attached thermode while no heat was applied for the same period of time. We hypothesized that there would be overlapping and distinct neural representations for each component (sensation, aversion, and location) of pain. To address the hypotheses, the contrasts generated at the second-level included (1) stimulated painful heat and non-painful warmth vs. all other conditions for the stimulation component, (2) stimulated painful heat and imagined painful heat vs. all other condition for the aversiveness component, and (3) body-site specific runs vs. all other runs for the location component. Significant activations were found for each component after family-wise error-correction at the $p < .05$ level. Heat pain specific activity was primarily centered on activity in the temporoparietal junction (TPJ), while warm stimulation activity featured a less intense and more distributed pattern. Imagination specific activity featured activation distributed across the brain with a cluster of activation centered within primary visual cortex (V1). Body-site specific activations consistently featured activity in three regions: TPJ, V1, and orbitofrontal cortex. Our results suggest that deep-phenotyping of pain components within an individual is possible and that common information areas such as body-site representations can be activated from a top-down (i.e., imagination) and bottom-up (i.e., thermal stimulation) pathway. This work increases the specificity of existing pain and cognitive signatures to differentiate activations across eight-body sites at the individual level, which has promising implications for the role of fMRI in precision medicine.

Disclosures: **M. Sun:** A. Employment/Salary (full or part-time):; Dartmouth College. **C.I. Bango:** A. Employment/Salary (full or part-time):; Dartmouth College. **K. Bo:** A. Employment/Salary (full or part-time):; Dartmouth College. **T.D. Wager:** A. Employment/Salary (full or part-time):; Dartmouth College.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.05

Topic: D.03. Somatosensation – Pain

Support: NIH/NIAMS R01-AR064367
NIH/NCCIH R01-AT007550, R61/R33-AT009306, P01-AT009965
NIH/NCRR P41-RR14075, S10-RR021110, S10-RR023043

Title: Prediction of cognitive behavioral therapy outcomes using baseline posterior cingulate cortical responses to pain catastrophizing in fibromyalgia

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Abstract: We found that the posterior cingulate cortex (PCC) encodes pain catastrophizing in fibromyalgia (FM) patients (Lee et al., 2018). Catastrophizing-linked PCC activity may be an important phenotypic factor that shapes long-term fibromyalgia outcomes; it is not clear, however, if PCC activation can also predict individual differences in clinical outcomes for psychological therapies targeting pain catastrophizing.

We enrolled 118 FM patients (all female, age=41.2±12.3), who were randomized into two treatment groups (cognitive behavioral therapy, CBT; and pain education, EDU). Patient-reported outcomes such as Pain Catastrophizing Scale (PCS) and Brief Pain Inventory (BPI) were collected before and after 8 weeks of therapy (PRE and POST, respectively). Neuroimaging (fMRI) data were collected prior to treatment during a cognitive task wherein patients engaged with catastrophizing (CAT) or neutral (NEU) statements, and instructed to reflect on the statements' applicability to their daily experience of FM pain. Univariate covariance analysis was performed to link baseline pain catastrophizing-specific 'CAT-NEU' brain activities to treatment effects (POST-PRE for PCS and BPI).

Of 118 FM patients, 82 patients finished PRE-therapy fMRI scans, reporting significant post-therapy improvements for PCS (change=-7.5±9.4, $P<0.00001$) and BPI (severity, change=-0.4±1.6, $P=0.029$) scores. The CBT group showed greater decrease in PCS compared to EDU groups, while the changes in both groups were significant (CBT=-8.9±9.3, $P<0.00001$; EDU=-4.6±9.1, $P=0.012$; $P_{CBTvsEDU}=0.049$). CBT group showed significant decrease in BPI (CBT=-0.6±1.5, $P=0.005$; EDU=0.0±1.7, $P=0.914$; $P_{CBTvsEDU}=0.12$) with no significant group difference. Greater pain catastrophizing-specific brain responses at baseline were correlated with less reduction in PCS scores in vPCC ($r=0.40$, $Z>3.1$; also significant for CBT group alone, $r=0.39$) and with less reduction in BPI severity in dPCC ($r=0.31$, $Z>2.3$).

We replicated previous findings linking vPCC activation with patient-reported catastrophizing and dPCC activation with fibromyalgia pain severity. We extend these findings by evaluating the predictive capacity of baseline PCC activation for FM treatment outcomes; greater baseline PCC activation during pain catastrophizing prompts was predictive of reduced benefit. This implies that baseline brain activity to pain catastrophizing may serve as an important predictive biomarker offers neural mechanisms how pain influences cognitive and emotional processes, and guide recommendations for better individualized treatment optimization.

Disclosures: J. Lee: None. **M.P. Berry:** None. **L. Isaro:** None. **A. Lazaridou:** None. **M. Paschali:** None. **A. Grahl:** None. **M.L. Loggia:** None. **D. Ellingsen:** None. **A.D. Wasan:** None. **R.R. Edwards:** None. **V. Napadow:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.06

Topic: D.03. Somatosensation – Pain

Support: NIH UO1NS077179
NIH UO1NS077352
NIH U54DA049116
NIH U54DA049115
NIH U54DA09113
NIH UM1NS112874
NIH UM1NS118922

Title: Multidisciplinary pain science in an age of biodiscovery: The Acute to Chronic Pain Signatures (A2CPS) program

Authors: *T. D. WAGER[1], A. KAHN[2], M. L. LINDQUIST[3], S. P. SUTHERLAND[3], E. BAYMAN[4], G. BERARDI[4], C. COFFEY[4], D. L. DAILEY[4], D. ECKLUND[4], L. FREY-LAW[4], K. A. SLUKA[4], C. G. T. VANCE[4], J. W. BURNS[5], A. BUVANENDRAN[5], J. JACOBS[5], X. J. ZHOU[6], C. BRUMMETT[7], A. CHANG[7], D. CLAUW[7], J. WALJEE[7], K. FISCH[8], T. HOWARD[9], J. JACOBS[10], K. JEPSEN[8], C. D. LANGEFELD[9], L. LAURENT[8], A. LOKSHIN[11], M. OLIVIER[9], W.-J. QIAN[10], A. C. P. S. CONSORTIUM[12];
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Abstract: Chronic post-surgical pain (CPSP) is defined as pain that develops or intensifies after surgery and persists more than 3 months excluding other causes of the pain (IASP definition). While most individuals recover from post-surgical pain within 6 to 12 weeks, an estimated 20 to 50 percent will experience persistent pain for months or years after the surgery. In addition to increasing the burden on patients and the health system, the need to manage intractable pain increases the risk for opioid-related adverse outcomes. There is an urgent need to identify factors (biomarkers) that can help predict who will undergo a transition from acute to chronic pain after surgery. Such biomarkers could inform the development of preventive and/or therapeutic targets, aid in diagnosis, and help monitor disease progression and treatment. Pain presents a particular challenge because its mechanisms span the central and peripheral nervous systems, and pain is modulated by the immune and endocrine systems. While innovative biomarkers have been developed in small-scale studies, large-scale discovery and evaluation of promising biomarkers will be required. The *NIH Common Fund Acute to Chronic Pain Signatures (A2CPS) Program* addresses this need. This landmark multidisciplinary longitudinal study aims to identify CPSP-related signatures, or patterns, made up of multiple biomarkers; we anticipate these signatures will lead to a better understanding of the neurophysiological mechanisms underlying CPSP and to the discovery of novel targets for the prevention and treatment of chronic pain. A2CPS will explore the factors that contribute to resilience and susceptibility to chronic pain after either total

knee replacement or thoracic surgery in 3,600 individuals across 10 clinical sites in the U.S. It will incorporate a comprehensive set of symptom-based, psychosocial, neuroimaging, genomic, proteomic, lipidomic, and metabolomic biomarkers prognostic of the transition to chronic pain. A2CPS combines a biomarker-validation approach to test carefully selected candidate biomarkers together with a discovery-science approach aimed at identifying new mechanistic causes of pain and therapeutic targets. Here, we describe the A2CPS study design, measures and biomarkers, along with the rationale for selecting them. We also describe the organization of the consortium and how it addresses the opportunities and challenges inherent in the large team-science paradigm. Finally, we describe future opportunities for the scientific community in accessing and utilizing the study's data and analytic resources.

Disclosures: T.D. Wager: None. **A. Kahn:** None. **M.L. Lindquist:** None. **S.P. Sutherland:** None. **E. Bayman:** None. **G. Berardi:** None. **C. Coffey:** None. **D.L. Dailey:** None. **D. Ecklund:** None. **L. Frey-Law:** None. **K.A. Sluka:** None. **C.G.T. Vance:** None. **J.W. Burns:** None. **A. Buvanendran:** None. **J. Jacobs:** None. **X.J. Zhou:** None. **C. Brummett:** None. **A. Chang:** None. **D. Clauw:** None. **J. Waljee:** None. **K. Fisch:** None. **T. Howard:** None. **J. Jacobs:** None. **K. Jepsen:** None. **C.D. Langefeld:** None. **L. Laurent:** None. **A. Lokshin:** None. **M. Olivier:** None. **W. Qian:** None. **A.C.P.S. Consortium:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 1P50DA044121-01A1
NSF GRFP DGE-1324585

Title: Signal or noise? Task-independent voxels can jointly decode painful tasks

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Abstract: Decoding mental states, such as pain, is a lofty goal in cognitive neuroscience. To decode, investigators commonly build and tune complex models using regions of interest; e.g., brain areas that correlate with a task of interest. We previously demonstrated that the voxels in these task-correlated areas are highly redundant for decoding—a random sample of 10% of a decoder's voxels performs just as well as using all of them. Here, we push this concept further by

systematically evaluating the decoding power of all voxels in the brain using the Neurosynth meta-analytic map for the term “pain”. We binned voxels by their absolute magnitude to create 10 decoders: the first (HIGH) had the greatest 10% of absolute voxel weights (each voxel is strongly correlated with “pain”) and the tenth (LOW) had the lowest 10% of absolute voxel weights (each voxel is nearly orthogonal to “pain”). Using 4 different datasets (total N=113), we evaluated the performance of the 10 decoders to discriminate pain from several different control tasks (visuomotor, touch (×2), vision, audition, and heat). Remarkably, three of the six conditions (visuomotor, touch, and vision) saw little-to-no degradation in discrimination performance (AUC) from HIGH to LOW. In the remaining tasks (touch, audition, and heat), AUCs decreased from HIGH to LOW by ~0.2, and only heat decreased to chance level. Despite the degradation in these latter conditions, this simple analysis powerfully reveals the presence of widespread information across the brain that can be leveraged for decoding. Linear combinations of voxels that should be nearly orthogonal to activation maps associated with pain contain enough information to decode acute pain from some, but not all, other stimuli.

Disclosures: A.D. Vigotsky: None. **R. Jabakhanji:** None. **M.N. Baliki:** None. **A. Apkarian:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant P50DA044121-01A1
NSF Grant No. DGE-1324585.

Title: The weak, inconsistent relationship between pain variability and relief in randomized clinical trials

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Abstract: Greater baseline variability is associated with greater pain relief in those who receive a placebo. However, it is unclear how generalizable this relationship is or if it is strong enough to be predictive. In this study, we analyzed data from two randomized clinical trials to add to the body of results from previous studies. Results differ between the two placebo groups: the placebo group in Study 1 showed a null correlation between baseline variability and response ($r(43) = 0.01$), whereas the placebo group in Study 2 showed a moderate, negative correlation

(r(33) = -0.43). Drug and no-treatment groups also showed differing correlations (Study 1: No Treatment: r(18) = -0.70; Study 2: No Treatment: r(12) = 0.23; Drug: r(35) = -0.35). When analyzed together, baseline pain variability was only able to account for 17% of variance in pain relief in Study 1 and 13% of variance in Study 2, leaving much variance unaccounted for. Even after controlling for other factors (such as baseline pain), the inability of baseline pain variability to account for substantial variance in pain response highlights that previously published results on the relationship between pain variability and treatment response are weak and inconsistent. Importantly, the relationship appears to be neither consistently specific nor sensitive to improvements in the placebo group, challenging claims in the literature. As a result, researchers and clinicians should not solely rely on using baseline pain variability as a determinant of treatment response but it may be useful to include as a covariate to improve analytical efficiency.

Disclosures: S. Tiwari: None. **A.D. Vigotsky:** None. **A.V. Apkarian:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.09

Topic: D.03. Somatosensation – Pain

Support: NIH/NINDS R01 NS112356-01 to DAS

Title: The interaction of cingulate cortex activity, pain catastrophizing, and cognitive performance while experiencing pain

Authors: *S. MARGERISON[1], A. FURMAN[1], S. KRIMMEL[2], M. KEASER[3], T. J. MEEKER[4], D. A. SEMINOWICZ[5];
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Abstract: Pain is a biopsychosocial phenomenon impacted by emotion and cognition. Pain catastrophizing is one factor contributing to the pain experience and is associated with increased pain levels and increased cognitive complaints among those with chronic pain. In addition, pain itself impacts cognitive function in those with chronic symptoms. We sought to determine how cognitive performance and pain catastrophizing relate to activity in the anterior midcingulate cortex (aMCC) and the subgenual anterior cingulate cortex (sgACC). Data from two existing fMRI datasets collected from healthy controls using a Siemens 3T Prisma MRI scanner with a 64 channel head coil were used. During the scans, participants performed the Multi-Source Interference Task (MSIT), a cognitive task developed to provide multiple sources of distraction. Our task included three levels of difficulty based on procedures by Seminowicz and Davis

(2007). This task was performed with and without pain and performance was measured by reaction time. Pain catastrophizing was assessed using the Pain Catastrophizing Scale (PCS). The effect of pain during the most difficult task on total BOLD signal of the sgACC and aMCC (n=24) and functional connectivity of the sgACC to the amygdala (n=12) and of the aMCC to the dorsolateral prefrontal cortex (dlPFC) (n=24) were calculated. We found that total BOLD signal at the aMCC was correlated with slower reaction time ($r = 0.491$, $p = .015$). There was also a non-significant negative correlation between PCS score and total aMCC activation ($r = -0.318$, $p = .130$). Total activation of sgACC during the pain condition was associated with slower response times ($r = .404$, $p= .050$). Our findings relative to the aMCC are consistent with prior studies. As the sgACC has been implicated in the regulation of stress and emotion, the association of sgACC total activation with slower response may indicate increased regulation needed to stay on task, resulting in reduced performance during the pain condition for those people. The relationship between cingulate activity, pain catastrophizing, and response time highlights the complex role the cingulate cortex plays in how pain affects cognitive function. Ongoing work will expand these findings to a larger population and further explore the role of the cingulate cortex in managing pain and task demands by using dynamic causal modeling to explore directionality of effect and expand connectivity analysis to more limbic structures, including insular cortex and hippocampus. Greater understanding of the complex relationship between pain and cognitive function is necessary for the successful treatment of those who experience chronic pain.

Disclosures: S. Margerison: None. **A. Furman:** None. **S. Krimmel:** None. **M. Keaser:** None. **T.J. Meeker:** None. **D.A. Seminowicz:** None.

Poster

P403. Pain Imaging and Perception II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P403.10

Topic: D.03. Somatosensation – Pain

Support: Bertha Rosenstadt Endowment Fund

Title: Trigeminal Nerve Abnormalities are Related to Pain Intensity in Myogenic Temporomandibular Disorders: a Probabilistic Tractography Study

Authors: *B. KAYA[1], I. CIOFFI[1], L. A. HENDERSON[2], M. MOAYEDI[1];
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Abstract: Myogenic temporomandibular disorders (mTMDs) are a painful condition affecting the muscles of mastication, causing mild to severe pain. In the past, mTMDs were traditionally thought to be idiopathic functional musculoskeletal pain disorders with no known etiology.

Recent evidence has shown that patients with TMD have abnormalities in the muscles of mastication, and the ascending trigeminal nociceptive system. However, there is mixed evidence on whether the microstructural organization of trigeminal nerve at the root-entry zone (REZ) into the pontine segment of the brainstem is abnormal in mTMD. A recent genome-wide association study has identified abnormalities in axon guidance genes in those with mTMD, indicating that perhaps the morphology or trajectory of the nerve may be abnormal. Probabilistic tractography allows for *in vivo* reconstruction of white matter tracts, and provides both qualitative and quantitative metrics of white matter trajectory and connectivity. Here, we aimed to compare the trajectory of the trigeminal nerve in patients with mTMD, compared to pain-free controls using probabilistic tractography. We investigated a previously collected cohort (Henderson Lab, University of Sydney) of chronic mTMD patients (5 M, 18 F; M = 43.86, SD = 14.03 years) and controls (5 M, 18 F; M = 47.51, SD = 14.84 years). 6 patients had pain on the right side, 1 patient had pain on the left, 12 patients had bilateral pain, and 4 patients did not report the side of pain. We delineated REZ and cisternal (CS) segments of the bilateral trigeminal nerves. Next, we performed probabilistic tractography in two directions: (1) from the CS in to the REZ (i.e., tracks going into the brainstem), and (2) from the REZ towards the CS (i.e., tracks going towards the face), to determine whether brainstem connectivity or nerve topography was abnormal in those with mTMD, respectively. We found that only the tracts towards the face of the right trigeminal nerve had significantly lower connectivity values in mTMD, compared to controls ($p<0.05$). This aligns with the most of the TMD cohort reporting right sided or bilateral pain. These abnormalities were significantly negatively correlated with self-reported pain intensity on the day of the MRI scan ($r= p<0.05$). This is the first study to perform tractography of the trigeminal nerve in chronic mTMD, and to identify trajectory abnormalities, in the absence of microstructural abnormalities. These data provide further evidence that chronic mTMD is associated with neuromuscular abnormalities.

Disclosures: B. Kaya: None. **I. Cioffi:** None. **L.A. Henderson:** None. **M. Moayedi:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.01

Topic: D.05. Olfaction and Taste

Support: ERC Grant 671251
FCT Grant 28509

Title: State predictions in primary olfactory cortex

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Abstract: Being able to predict future states of your environment based on past experience is a powerful tool for adaptive behavior. Several theories exist that formalize the use of predictions for behavior and neural coding. In Bayesian frameworks, predictions take the form of priors, and are combined with incoming sensory information. In predictive coding frameworks, sensory signals are compared with a generative internal model to produce prediction error signals. While behavioral support for these theories is strong, the evidence of their implementation in neural circuits is controversial. To study the effects of predictability on sensory neural codes, we combined odor-based virtual reality with large-scale chronic electrophysiology in head-fixed mice. We recorded neural responses in primary olfactory cortices while paired odors were presented sequentially. While primary responses to odors have been well characterized in these cortices, modulation of those responses when predicted has received little focus. We presented odor pairs with a brief temporal separation. The first odor predicted the second odor and on a small subset of trials, the predictive odor was omitted to assess the responses to the second odor without the prior. On the single cell level, responses to the second odor were significantly modulated by the presence of the predictive first odor. Separate subsets of cells showed increases and decreases in response magnitudes. Across sessions and animals, the average response modulation was close to zero, suggesting that the predictive first odor acts as a prior that rotates the second odor representational space without changing the overall population firing rate. This shows that the prior modifies the odor representation. Surprisingly, some cells started responding to the second odor before its onset given the predictive first odor. The onset of the second odor response was also earlier on the population level. Linear decoders showed increased performance in decoding the first odor stimuli before their onset when predicted. Thus, the prior is explicitly represented before the onset of the second odor. Our data show that odor representations in primary olfactory cortices are powerfully modulated by sensory prediction, and suggest that priors are represented as rotations of the primary representation space.

Disclosures: T. Stensola: None. **H. Stensola:** None. **M. Patwa:** None. **E. DeWitt:** None. **Z.F. Mainen:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.02

Topic: D.05. Olfaction and Taste

Support: NIH Grant 5R01DC014487

Title: Cortico-bulbar feedback supports behavioral flexibility during rule-reversal

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Abstract: To survive, animals flexibly adjust their actions to relevant changes in the world. Mice are expert at recognizing odorants in ever-changing sensory scenes. Yet, little is known about: (1) how odorant representations are modified upon sudden changes in stimulus contingency and (2) how changes in feedforward and feedback odorant representations causally relate to behavior. The piriform cortex (PC) receives input from both the olfactory bulb (OB) and association areas (e.g. orbitofrontal cortex). Furthermore, the PC sends rich top-down feedback and controls the activity of the OB outputs, in a cell-type specific manner. It is, thus, ideally positioned for integrating sensory input and experience-based sensorimotor predictions.To determine whether the cortico-bulbar feedback supports behavioral flexibility, we trained mice in a Go/No-Go rule-reversal task (>85% accuracy) and monitored the activity of feedback boutons (GCaMP5/7b vs. GFP controls) across bulb layers via multiphoton microscopy (n = 12 mice; 53 sessions). Within the same session (~270 trials), the reward contingency was switched (4 reversals) across blocks of contiguous trials (~45), rewarding either the odor or sound cue. To disambiguate reward-related signals from signatures of motor activity, in a subset of experiments, a 500 ms delay was imposed between the cue offset and the reporting period. Cortical feedback boutons showed dense responses (55% responsive boutons vs ~4% in naïve animals) aligned to specific trial events. Strikingly, individual boutons mirrored closely the block-structure of the task and changed, within seconds, their response kinetics, shape, and amplitude to the same sensory cue, following each rule-reversal. Cortical feedback matched the *perceived* current reward-rule and slightly lagged in updating, similarly to the animal's behavior. We trained and cross-validated classifiers (multi-layer perceptrons) to decode task features ranging from stimulus identity and trial contingency to behavioral outcome. The classifiers' performance for each variable rapidly increased during cue-delivery and peaked during the delay epoch. The performance remained high throughout lick-reporting, and for several seconds after reward collection. In ongoing experiments, we are assessing the effects of optogenetically suppressing the cortical-bulbar feedback on the rule-switching behavior and on OB output activity.Thus, the cortical-bulbar feedback carries information related to stimulus identity, contingency, and behavioral outcome, which is readily re-formatted across different rules of engagement in evolving environments.

Disclosures: D.E. Hernandez: None. **A. Ciuparu:** None. **P. Garcia da Silva:** None. **M.C. Velasquez:** None. **B. Rebouillat:** None. **M. Gross:** None. **M.B. Davis:** None. **R.C. Muresan:** None. **D.F. Albeanu:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.03

Topic: D.05. Olfaction and Taste

Support: NIH grant R34NS116731
NIH fellowship F32MH118724

Title: Neural correlates of time and place in the olfactory bulb of freely-moving mice

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Abstract: Odors provide useful navigational and episodic information, but however numerous the odorant receptor genes in an animal's genome, there is no receptor for time or place. To optimally orient by olfactory information, brains must incorporate odor-driven activity into representations of self-movement and -location. While a feedforward model might predict that such multimodal integration would be implemented in "high-level" areas, recent studies in other sensory modalities demonstrate that motor- and location-related signals are common in primary sensory areas. Motivated by these findings, and given the strong reciprocal connection between olfactory system and hippocampus, we hypothesized that the olfactory bulb might also encode navigational information. To test this hypothesis, we captured the sniffing and movement of mice while recording spiking activity in their olfactory bulb, in the absence of experimenter-applied stimuli or tasks. We first compared breathing and spiking between head-fixed and freely-moving states. During head restraint, sniffing is arrhythmic -- intersniff intervals are broadly distributed. In striking contrast, without restraint respiration is organized into discrete rhythmic states lasting minutes. Intersniff intervals are more metronomic and inversely proportional to movement speed. In the bulb, we found that many units fired preferentially at characteristic times after inhalation, as previously reported. In addition, many units fired preferentially at characteristic sniff frequencies, particularly during free movement, often in correspondence with the rhythmic states of sniffing. In addition to these time-selective signals, we also found that many olfactory bulb neurons are place-selective during free movement. To test whether this position-selective activity carries usable information about the mouse's location, we decoded position from small ensembles of 20-50 simultaneously recorded units. We could decode the mouse's position with above-chance accuracy in all sessions. Thus, even during spontaneous behavior and ambient stimuli, information about time and place can be read out from the activity of the olfactory bulb. A mouse needs to know where and when it encountered different smells: olfactory information must be contextualized and incorporated into cognitive maps of environment and self. Our results suggest that this contextualization process begins as soon as olfactory information enters the brain.

Disclosures: M.A. Brown: None. **T. Findley:** None. **S.C. Sterrett:** None. **A.P. Weible:** None. **M. Karlsson:** None. **A.L. Fairhall:** None. **J.M. Murray:** None. **M.C. Smear:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.04

Topic: D.05. Olfaction and Taste

Support: NIH grant DC005259
Brain Science Institute, Korea Institute of Science and Technology

Title: Configural responses to odor mixtures in head-fixed mice

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Abstract: Odor mixtures can exhibit configural (the mixture is considered to have an odor different from any of the individual component odorants) or elemental (the components in a mixture are recognizable) properties. However, it remains unclear whether the olfactory bulb, the brain structure that mediates the first stage of olfactory information processing, might contribute to the configural perception of certain odor mixtures. The aim of the present study was to compare elemental and configural perception of odor mixtures in head-fixed mice in lick/no-lick discrimination task. In this study we used a binary odor mixture (citronellal-octanal) that elicited configural responses in freely moving rats (Kay et al, (2003). We followed a procedure for motivating mice by limiting their access to water as was initially described by Guo et al, (2014). All mice undergoing water restriction were monitored daily for hydration, weight, ruffled fur, and movement. The head-fixed mice were trained in a lick/no-lick discrimination task where animals were rewarded with water for licking in response to binary citronellal-octanal mixtures of two different ratios, 1:1 and 3:1 (citronellal-octanal). Water was not available for trials with the non-rewarded odor (hexanol) and licking on those trials was punished with a longer inter-trial interval. Licking was signaled by mice breaking a light path in a Bpod r2 water port with their tongue. All mice learned to avoid licking in no-lick trials during the first session and reached a learning criterion (60% correct responses) within 100 - 220 trials. To determine whether the perception of the odor mixtures is elemental or configural we compared the response to the mixture, the individual components, and a novel odor (isopentyl acetate). The response to the individual components and the novel odor were similar to each other and were different from the response to the mixture and to hexanol, showing that the mixture perception was configural.

Disclosures: D. Klymyshyn: None. **L.B. Cohen:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.05

Topic: D.05. Olfaction and Taste

Support: NIDCD R01 DC014367
DARPA/MTO HR0011-18-2-0024

Title: Effect of trigeminal input on binary odor mixture perception

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Abstract: The experience of smell can be modulated by the trigeminal system and its interactions with the olfactory system, but how chemesthesis in the nose interacts with olfactory perception is not fully understood. Trigeminal nerve endings in the olfactory sensory epithelium are stimulated by most odorants and activate cell bodies in the trigeminal ganglion. Neither the behavioral effects nor the underlying mechanisms of their interactions have been systematically characterized. Almost all odorants stimulate the trigeminal nerve, some even at low concentrations. Thus, it is reasonable to postulate that the influence of trigeminal input is almost always at play in odor perception, especially for odor mixtures, and most natural odorants are blends of many different monomolecular odorants. From the standpoint of olfactory psychophysics, there is also no satisfactory theory that predicts the perceptual qualities of even a binary mixture, and some of the confounds in prior approaches may come from ignoring the role of trigeminal-olfactory interactions. To address how trigeminal activation may play a role in odor mixture perception, we trained rats to discriminate 4 different pairs of monomolecular odorants of varying relative trigeminal intensities and profiles, and asked them which of the two odorants a binary mixture smells like while varying ratios of the two odorants systematically. We predicted that comparative trigeminal intensities of component odorants would help explain overshadowing. All odor mixtures tested showed some degree of overshadowing of one component by the other, when we accounted for differences in volatility. The magnitude of difference in component trigeminal intensities did not predict the degree of overshadowing in the binary mixtures we tested. However, the data do suggest that the component of higher trigeminal intensity is overshadowed by the trigeminally weaker odorant in a binary mixture. To better inform a systematic comparison of trigeminal profiles of odorants used, we also recorded and compared electrophysiological responses in the olfactory bulb and trigeminal ganglion under anesthesia with the same odorants used for behavioral analysis.

Disclosures: H. Li: None. **C. Lee:** None. **Z. Leveroni:** None. **S. Mislitchi:** None. **L.M. Kay:** None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.06

Topic: D.05. Olfaction and Taste

Support: In-House Grant from NYIT COM

Title: Odor identification in novel olfactory environments is selectively impaired in a mouse model of autism

Authors: G. H. OTAZU;
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Abstract: Aversion to novel stimuli in autism affects quality of life. We developed a behavioral paradigm to study the effect of novel background odors on odor discrimination in a mouse model of autism. We trained wild type mice to discriminate target odors in known background odors. When tested, WT mice could discriminate known targets in novel background odors, a task similar to the visual CAPTCHA used to distinguish humans from computers. Using glomerular intrinsic imaging data, we showed that WT mice used an algorithm that required less training data than a linear classifier or nearest neighbor classifier. The *Cntnap2*$^{-/-}$ mouse model of autism learned simple odor discriminations at the same rate as WT mice. *Cntnap2*$^{-/-}$ mice were also able to learn to identify odors in weak backgrounds as well as WT mice and CNTNAP2 knockout mice matched WT mice performance in the presence of known backgrounds. However, performance fell almost to chance levels in the presence of novel backgrounds. WT mice use a robust algorithm for detecting odors in novel environments and this computation is selectively affected in a mouse model of autism.

Disclosures: G.H. Otazu: None.

Poster

P426. Olfactory Coding and Perception

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P426.07

Topic: D.05. Olfaction and Taste

Support: R01DC015426

2T32MH067564
T32NS047987

Title: Dimensionality of perceptual odor representations increases across the central olfactory pathways

Authors: *V. SAGAR, T. KAHNT;
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Abstract: The central olfactory system transforms chemical information of odor molecules into a unified olfactory percept. Previous work suggests that the piriform cortex (PirC) supports odor perception, whereas the orbitofrontal cortex (OFC) is involved in higher-order olfactory cognition. However, the nature and dimensionality of odor encoding in these areas has not yet been determined. Addressing this question requires mapping neural responses to a large number of odors. Here we combined computational modeling with a high-precision functional mapping approach to examine the encoding of odors in different olfactory areas. We collected functional magnetic resonance imaging (fMRI) responses to 160 monomolecular odors from individual subjects (18 h of fMRI hours per subject, N=3). In a first step, we used representational similarity analysis to quantify the unique contribution of chemical structure and perceptual quality to neural response patterns. The difference between the represented perceptual and chemical information was significantly larger in the OFC compared to the PirC and amygdala, with perceptual quality emerging as the dominant driver of odor-evoked responses in the OFC. Moreover, we used voxel-wise encoding models to determine the specific perceptual features encoded in these areas. We found that the dimensionality of the perceptual feature space encoded in neural responses differed significantly across olfactory areas, with a simple and low-dimensional representation in PirC, and complex and high-dimensional perceptual feature spaces in the OFC. These findings challenge the notion that odor perception occurs at the level of PirC, and suggest that OFC plays a key role in supporting a rich and multi-dimensional representation of our olfactory environment.

Disclosures: V. Sagar: None. **T. Kahnt:** None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01 DC 000141

Title: Pharmacological manipulation of the apical cochlear amplifier reveals unique mechanical encoding properties for encoding sound

Authors: *G. W. S. BURWOOD[1], A. L. NUTTALL[1], A. FRIDBERGER[2];
[1]Oregon Hearing Res. Ctr., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Biomed. and Clin. Sci., Linkoping Univ., Linkoping, Sweden

Abstract: The apex of the mammalian cochlea is the location where speech and music frequency sounds are transduced into auditory nerve impulses. Recent reports have highlighted the fundamental differences between not only the mechanics of the cochlear apex and high frequency base, but also that of the basilar membrane and reticular lamina at each location. Using data obtained by our minimally invasive guinea pig preparation and our spectral domain optical coherence tomography system, we describe the relative motion of components within a single midmodiolar plane the apical cochlea, showing the organ of Corti from three locations from an area comprising the final 20% of the cochlear spiral. The displacement, phase and inter-location group delay in response to single and multi-tone stimuli are reported. Preparations were given IP furosemide, a loop diuretic agent which abolishes endocochlear potential. Endocochlear potential is known to be required for maximum force generation by electromechanical somatic motility outer hair cells. Measurements were made before and during exposure to furosemide, and post mortem. Overall cochlear sensitivity was monitored using gross neural threshold response at the round window, to a 2 kHz tone. In this way, we elucidate the outer hair cell force dependent component of transverse organ of Corti motion, and assess how it changes with distance to the helicotrema. We found that the cutoff frequencies of the three iso-intensity tuning curves measured from the reticular lamina at these cochlear locations were closer than has been predicted by tonotopic mapping of the cochlea. In fact, the most apical region did not display the lowest cutoff frequency - contrary to expectations. Additionally, we found that the average group delay difference between two locations separated by one half cochlear turn is minimized in vivo. This phase delay minimization was abolished by furosemide and following euthanasia, and was not affected in preparations that did not respond to IP furosemide. Our data also show that signal processing mechanisms within the cochlear apex may emerge partially from the gradual change in amplification capacity and nonlinearity with respect to distance from the helicotrema, altogether suggesting an emphasis on temporal sensitivity in a distributed system, over place coding.

Disclosures: G.W.S. Burwood: None. **A.L. Nuttall:** None. **A. Fridberger:** None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.02

Topic: D.06. Auditory & Vestibular Systems

Title: Otolith Endorgans Hair Cell Polarity-Specific Segregation of Primary Vestibular Afferent Fibers to the Brainstem and Cerebellum in Chick Embryo

Authors: W. GHERMAN[1], K. REJENT[2], A. BHAVSAR[2], P. W. FRANK[3], D. GIOVANNUCCI[2], *A. MAKLAD[4];
[2]Neurosciences, [3]Med. Educ., [1]Univ. of Toledo, Toledo, OH; [4]Neurosciences, Univ. of Toledo, Perrysburg, OH

Abstract: Perception of linear acceleration and head position relative to the line of gravity pull is the function of the utricle and a saccule in mammals. One of the most conserved features of the vestibular system of all vertebrates is the morphological polarity of the hair cells. In all vertebrate, the otolith endorgans hair cell morphological polarity is reversed across a line known as striola. The function of this polarity reversal at the striola is currently unknown. In mammals, one polarity domain outside the striola was found to project almost exclusively to the cerebellum, whereas the other polarity inside the striola projects almost exclusively to the brainstem. The current study is designed to test whether the central projection of the different polarity domains in the otolith endorgans in other classes in vertebrate: Aves. Lipophilic retrograde tracers (NeuroVue Red and NeuroVue Maroon) were implanted in the vestibular nuclei and the nodulus and uvula of the cerebellum, respectively, in post-fixed pre-hatching chick embryos. After the diffusion time was complete, the maculae of otolith endorgans were dissected out and examined with confocal microscopy. In the macula of the utricle, Neuro Vue Red placed in the vestibular nuclei retrogradely labeled a distinct domain of hair cell located inside the striola region, whereas the NeuroVue Maroon retrogradely labeled hair cells located outside the striola. Similarly, in the macula of the saccule, NeuroVue Red implanted in the vestibular nuclei labeled an area of the hair cells located inside the striola region, whereas, NeuroVue Maroon labeled an area of the hair cells outside the striola. No area of overlap or double labeled cells were seen in the different domains. Our data suggest that the central segregation of the primary otolith afferent of the different polarity domain reported previously in mammals is also conserved in other classes such as Aves.

Disclosures: W. Gherman: None. **K. Rejent:** None. **A. Bhavsar:** None. **P.W. Frank:** None. **D. Giovannucci:** None. **A. Maklad:** None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC017147
RNID international research grant

Title: Functions of GRXCR2 in hearing and deafness

Authors: *B. ZHAO;
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Abstract: Stereocilia of cochlear hair cells are specialized mechanosensing organelles that convert sound-induced vibration to electrical signals. The base of the stereocilia, which is the site of stereocilia pivoting, is organized by several proteins necessary for human hearing and essential for stereocilia morphogenesis and function. GRXCR2, localized at the base of stereocilia, has been found to be critical for the morphogenesis of stereocilia through its interaction with taperin, an actin regulatory protein also concentrated at the base of stereocilia. Rescuing the morphological defects of stereocilia in *Grxcr2*-null mouse by reducing taperin expression level only partially restores its hearing, suggesting that GRXCR2 interacts with some other proteins required for auditory perception. Recently, we identified a novel interaction between GRXCR2 and CLIC5. Disrupting their interaction *in vivo* has minimal effects on stereocilia morphogenesis but leads to moderate hearing loss at lower frequencies and severe hearing loss at higher frequencies, strongly suggesting that the interaction between GRXCR2 and CLIC5 is essential for normal hearing. Thus, our study provides a foundation for future investigations of the functions of a protein complex in the basal region of stereocilia critical for sound perception.

Disclosures: B. Zhao: None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH grant R01 DC012347 (RAE, AL)
NSF GRFP (HRM)

Title: The potassium channel subunit Kv1.8 (Kcna10) differentially shapes tuning, gain, and response latency of type I and II vestibular hair cells

Authors: *H. R. MARTIN[1], S. D. PRICE[2], A. LYSAKOWSKI[2], R. A. EATOCK[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Dept. of Anat. & Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Vestibular hair cells (HCs) convert head motions into receptor potentials that drive downstream gaze and postural reflexes, spatial navigation, and gravity sensation. In amniotes, type I and II HCs (HCI and HCII), express large K^+ conductances with different voltage dependence and inactivation, which differentially shape receptor potential gain, tuning, and timing. We investigated the roles in HCIs and HCIIs of $K_V1.8$, an understudied channel encoded by the Kcna10 gene. $K_V1.8$-null animals lack vestibular evoked potentials normally evoked by transient head motions (Lee et al, 2013). We stimulated HCs with voltages, currents, or hair bundle deflections and recorded whole-cell currents and voltages from HCs in intact utricles from $K_V1.8$-null mice and wildtype (WT) and heterozygous littermates (postnatal days 5-279). We show that $K_V1.8$ is necessary for the dominant but very different K^+ conductances in HCI and HCII, and that these K^+ conductances differentially affect response gain, tuning, and timing.
$K_V1.8^{-/-}$ HCIs lacked the large, low-voltage-activated, non-inactivating K^+ conductance ($g_{K,L}$, V_{half} = -85 ± 2 (SE) mV) specific to mature WT HCI. The residual outward rectifier (V_{half} = -41 ± 1 mV) was partly blocked (47 ± 13 %) by the K_V7 blocker, XE991. The input resistance of $K_V1.8^{-/-}$ HCIs was 20-fold greater than that of WT HCIs. As a result, the receptor potential evoked by hair bundle deflections increased in size but slowed, producing a lower low-pass corner frequency (~20 Hz vs. ~400 Hz) and greater phase lag.
$K_V1.8^{-/-}$ HCIIs lacked the fast-inactivating, α-DTX-sensitive A-type conductance (g_A, V_{half} = -34 ± 1 mV) typical for WT HCII. The small residual conductance (V_{half} = -32 ± 1 mV) was a non-inactivating delayed rectifier. Without g_A, the membrane potential evoked by current steps was more likely to show electrical resonance, had longer latency, and recovered more slowly after depolarization. In response to sinusoidal mechanical stimulation, the $K_V1.8^{-/-}$ HCII receptor potential had a significantly lower best frequency (8 ± 2 Hz vs 17 ± 4 Hz) and greater phase lag above 5 Hz.
Thus, $K_V1.8$ is necessary for two distinct conductances ($g_{K,L}$ and g_A) in neighboring HCs. Immunocytochemistry localizing $K_V1.8$ to the basolateral membranes of HCIs and HCIIs and other evidence that it is conductive (Lang et al, 2000; Dierich et al, 2020) suggests that $K_V1.8$ is a pore-forming subunit of both $g_{K,L}$ and g_A. $K_V1.8$ also contributes to a non-inactivating outward rectifier (V_{half} = -2.2 ± 0.4 mV) in cochlear inner HCs (Dierich et al, 2020). $K_V1.8$ may partner with other K_V1 subunits in the different HC types or be subject to other forms of modification.

Disclosures: H.R. Martin: None. **S.D. Price:** None. **A. Lysakowski:** None. **R.A. Eatock:** None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.05

Topic: D.06. Auditory & Vestibular Systems

Support: NIH-NIDCD R01 DC012347

Title: Nonquantal transmission at the vestibular hair cell-calyx synapse

Authors: *A. GOVINDARAJU[1], I. H. QURAISHI[2], A. LYSAKOWSKI[3], R. EATOCK[4], R. RAPHAEL[1];
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Abstract: Vestibular hair cells transduce head movement and inform neural pathways that control gaze, balance and orientation. In mammals, birds and reptiles, vestibular afferent neurons form a uniquely shaped synaptic terminal, termed a calyx, around vestibular type I hair cells. This arrangement supports nonquantal transmission (NQT), a neurotransmitter-independent mechanism of coupling changes in hair cell potential to changes in calyx potential. The existing view is that a large low-voltage-activated potassium conductance in the hair cell (gKL) drives potassium accumulation in the synaptic cleft and depolarizes the calyx membrane. However, a full biophysical model of NQT has not been developed and the role of other aspects of the system remain unclear, including the role of ion channels present on the calyx inner face membrane (Kv7, HCN) and the possibility of changes in electrical potential within the synaptic cleft space. Here we present a computational model of NQT that integrates existing experimental observations into a coherent picture. We show that changes in electrical potential in the synaptic cleft are necessary to explain fast post-synaptic currents that were recently observed. Changes in electrical potential in the cleft advance the phase of the calyx response, facilitating faster synaptic transmission of vestibular stimuli than can be explained with potassium accumulation alone. Together, both processes may contribute to the mechanistic basis for the speed of vestibular reflexes which involve peripheral vestibular afferents. The theoretical approach we have developed may be further extended to investigate neurotransmitter-independent modes of synaptic communication in other systems such as the retina.

Disclosures: A. Govindaraju: None. **I.H. Quraishi:** None. **A. Lysakowski:** None. **R. Eatock:** None. **R. Raphael:** None.

Poster

P430. Hair Cells and the Periphery: Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P430.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01 DC012347
HHMI Gilliam Fellowship for Advanced Study

Title: Persistent and resurgent sodium currents enhance spiking excitability in mouse vestibular ganglion neurons.

Authors: *S. BAEZA LOYA, R. A. EATOCK;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: The vestibular inner ear transmits information to the brain via two populations of primary vestibular ganglion neurons (VGNs) which differ in the regularity of action potential (AP) timing. Studies of isolated VGNs have shown that irregular neurons are less excitable than regular neurons, producing transient and sustained spiking in response to current steps, respectively. Although voltage-gated sodium (Na_V) currents drive the rising phase of APs, their contributions to excitability and regularity differences are not fully understood. Na_V currents in VGNs are dominated by transient (inactivating) currents but can include persistent (noninactivating) and/or resurgent currents (which flow after relief from inactivation block). Here we consider the impact of different Na_V current modes on patterns of AP firing.
Whole-cell recordings were taken from mouse VGNs (postnatal days, (P)3-25) isolated and cultured overnight. In a sample of 78 VGNs, all had large transient Na_V currents blocked by 1 μM TTX; 42 had persistent current (P3-25), 6 had resurgent current (P11-20), and 4 (P11-20) had both persistent and resurgent currents. Application of $Na_V1.6$ blocker 4,9-anhydro-tetrodotoxin (4,9-ah-TTX) partially blocked all three current modes, indicating that a substantial portion of each is carried through $Na_V1.6$ channels. In current clamp, application of 4,9-ah-TTX decreased neuronal excitability: increasing current threshold for spiking in all VGNs and decreasing AP rate in sustained (regular) VGNs.
We lack ways to experimentally isolate the effects of transient, persistent, and resurgent currents on spiking because they flow through the same channel subunits. Therefore, we adapted a conductance-based VGN model with transient Na_V current (Hight and Kalluri, J Neurophysiol 116:503, 2016), adding persistent and resurgent currents (Venugopal et al., PLoS Comput Bio 15(6): e1007154, 2019) and adjusting expressions for properties measured in our VGNs. The addition of persistent and resurgent current components had a negligible effect on firing by the model transient (irregular) VGN. For the model sustained (regular) VGN, adding persistent and resurgent currents decreased first-spike latency (delay relative to stimulus onset) and increased spike rate.
These results suggest that increasing Na_V channel availability in the after-spike interval with persistent and resurgent currents may enhance excitability and hence regularity of regular VGNs. The difference in regularity may represent distinct encoding strategies, with regular afferents using a rate code and irregular afferent using a temporal code. Supported by NIDCD R01 DC012347 and HHMI Gilliam.

Disclosures: S. Baeza Loya: None. **R.A. Eatock:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant K99DC018600
NIH Grant R01DC011284

Title: Transformation of acoustic information to sensory decisions in parietal cortex

Authors: *J. D. YAO, K. O. ZEMLIANOVA, D. H. SANES;
New York Univ., New York, NY

Abstract: The ability to integrate sensory information over time - often called temporal integration - is fundamental for guiding perceptual decisions. Perceptual decision-making has been studied at the level of stimulus representations in sensory cortex, and in higher cortical areas that are thought to produce premotor decision-related signals. Here, we ask how the neural representations of sensory information are transformed in the auditory-recipient parietal cortex, a region that is causally associated with sound-driven perceptual decisions (Yao and Sanes, 2020). To examine this, gerbils were first trained and tested on an appetitive alternative forced-choice (AFC) auditory temporal integration task. Specifically, gerbils were required to discriminate between amplitude modulated (AM) noise at 4 versus 10 Hz across a range signal durations (100-2000 ms). Task performance was poor at very short AM durations (100-300 ms), improved with longer durations, and reached an optimum at ~600-800 ms. Our previous work revealed a causal relationship between this behavioral read out of auditory evidence accumulation and the activity of auditory cortical projections to parietal cortex (Yao and Sanes, 2020). To determine how a parietal cortex encoding mechanism could support the behavioral result, we conducted wireless recordings of single-unit activity from neurons in parietal cortex while gerbils performed the auditory task and while they were disengaged from the task (i.e., passive listening to the identical acoustic stimuli). During task performance, individual neurons displayed a sharp rise in firing rate that peaked prior to the animals' decision. To evaluate the read out of population activity, we used a linear classifier procedure that applies a support vector machine algorithm to decode 4 versus 10 Hz stimulus conditions from simultaneously recorded parietal cortex neurons during each session. Decoder performance during task performance was strongly correlated with behavioral outcomes across stimulus conditions, leaving open whether the parietal representation was purely motor or included a sensory component. To address this, we evaluated decoder performance during disengaged sessions, and found a representation of the AM rate across a range of signal durations. Our findings suggest that parietal cortex neurons represent both the acoustic stimulus and sound-driven decision, demonstrating the progression of auditory processing downstream of auditory cortex.

Disclosures: J.D. Yao: None. **K.O. Zemlianova:** None. **D.H. Sanes:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.02

Topic: D.06. Auditory & Vestibular Systems

Support: Simons Foundation Grant 542977SPI

Title: Sensitivity to unpredicted song perturbations in an avian auditory cortex

Authors: *M. T. L. HAPP, A. BAHLE, M. FEE;
MIT, Cambridge, MA

Abstract: In order to navigate their environments, intelligent agents must detect when stimuli are novel and surprising. Unpredictable stimuli are important for (1) recognition and learning of novel stimulus patterns and (2) learning from outcomes that differ from agents' expectations or goals. These two key aspects of novelty detection are illustrated by songbirds learning to sing, making songbirds excellent model organisms for the study of novelty detection.
In the song learning process, juvenile birds must (1) form a memory of a tutor song and (2) detect deviations from that learned template in the juvenile bird's own renditions. The first step requires recognition and learning of a novel stimulus pattern (the tutor song), and the second requires detection of deviation (in the bird's own rendition) from a familiar template (the internalized tutor song). What brain circuits underlie these processes? A secondary auditory area known as the caudomedial nidopallium (NCM) is believed to be important for the formation of the tutor song memory, and it is well known that neurons in this region habituate in a rapid and long-lasting manner to novel songs (Chew et al., 1995). This phenomenon, known as stimulus-specific adaptation (SSA) has been proposed to be crucial for the formation of memories for novel songs (including the tutor song). Once this memory is formed, it can serve as a template with which the young bird's developing song can be compared. However, the comparison circuit has not yet been identified.
As a clue in this search for a comparison circuit, we report the discovery of neurons in NCM that are sensitive to acoustic perturbations (e.g. white noise bursts and gaps of silence) to familiar songs, but not to identical perturbations of the same song before repeated exposure. Recordings were made using neuropixels probes to record from hundreds of single and multi-units in NCM of the zebra finch (Taeniopygia guttata). Birds were head-fixed and presented with familiar and perturbed auditory stimuli. We propose that the stimulus-specific adaptation and deviance detection may work in tandem to subserve the song learning process, first by song memory formation and then by detection of deviation from a learned template.

Disclosures: M.T.L. Happ: None. **A. Bahle:** None. **M. Fee:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.03

Topic: D.06. Auditory & Vestibular Systems

Support: T32-MH019524
1R01-DC018802

Title: Expectation of self-generated sounds drives predictive processing in mouse auditory cortex

Authors: *N. AUDETTE[1], W. ZHOU[2], D. M. SCHNEIDER[2];
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Abstract: Sensory stimuli are often predictable consequences of one's actions, and behavior exerts a correspondingly strong influence over sensory responses in the brain. Closed-loop experiments with the ability to control and perturb the sensory outcomes of specific animal behaviors have revealed that neural responses to self-generated sounds are suppressed in the auditory cortex, suggesting that prediction may play a critical role in local sensory processing. However, it is unclear whether this phenomenon derives from a precise movement-based prediction, if the cortex can support local implementation of predictions, or how it affects the neural representation of incoming stimuli. We address these questions by designing a behavioral paradigm where mice learn to expect the predictable acoustic consequences of a simple forelimb movement. Dense neuronal recordings from auditory cortex revealed suppression of neural responses that was strongest for the expected tone during a narrow time window surrounding the specific sound-associated movement. Predictive suppression was concentrated in layers 2/3 and 5, preceded by the arrival of movement information in deep layers, and driven by motor-sensory coupling rather than task engagement, behavioral relevance, or reward association. Despite large-scale suppression at the population level, the auditory cortex retained a sparse representation of expected self-generated sounds, while a substantial population of auditory cortex neurons in L2/3 and L5 encoded prediction errors. These findings illustrate that expectation, learned through motor-sensory experience, drives layer-specific predictive processing in the mouse auditory cortex.

Disclosures: N. Audette: None. **W. Zhou:** None. **D.M. Schneider:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH P50 HD103555 (Cam Core)
NIH P50 HD103555 (Research Project)

Title: A pupillometric assessment of pre-pulse inhibition in sensory gating in mice

Authors: *S. JOSHI[1], S. SMITH[1], Z. H. MRIDHA[1], J. DEGEE[1], D. CONNOLLY[2], J. HOLDER[3], R. C. SAMACO[4], M. J. MCGINLEY[1];
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Abstract: Pupil size is widely used as a non-invasive metric of brain state. Previous studies have shown links between pupil size and the activity in major neuromodulatory systems such as the locus coeruleus norepinephrine (LC-NE) and basal forebrain acetylcholine (BF-ACh) systems. However, evoked or baseline changes in pupil size and its relationship with the activation of such systems under different perceptual or behavioral conditions remains poorly understood. Here, we test the use of pupillometry in measuring sensory information gating in the auditory system. Using a novel paradigm in head-fixed mice, we measured the response of the pupil to a loud tone when presented alone and when preceded by a weaker tone – a prepulse. Since no behavioral response is required in this paradigm, pupil and neuromodulatory responses are less confounded by motor activity than in behavioral tasks.
We present our preliminary findings showing attenuation of pupil responses by the prepulse and changes in neuromodulator levels due to tone alone and tone plus prepulse. To test whether such pupil indexed sensory gating is affected by neural dysfunction, we focused on the *MECP2* and *SHANK3* genes that are involved in neural development and synapse function. Mutations in these genes are associated with autism spectrum disorders (ASD). We measured pupil responses in *MECP2* and *SHANK3* mutant mice and found reliable difference in both baseline and evoked pupil size compared with wild type littermates.
Since pupil responses are sensitive to the sound level and to the presentation of a prepulse, these findings provide a link between pupil size and attentional blink – the attenuation of sensory processing while a previous stimulus is being processed. Our findings also show that pupil measurements are sensitive to the effects of specific genetic mutations associated with ASD on sensory information gating. In ongoing experiments, we are probing the neural circuit basis of these effects.

Disclosures: S. Joshi: None. **S. Smith:** None. **Z.H. Mridha:** None. **J. deGee:** None. **D. Connolly:** None. **J. Holder:** None. **R.C. Samaco:** None. **M.J. McGinley:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.05

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC018055-01

Title: Predictive coding in songbird secondary auditory cortical neurons

Authors: *S. RUDRARAJU[1], B. H. THEILMAN[2], T. GENTNER[3];
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Abstract: Predictive coding (PC), a theoretical framework in which the brain compares a generative model to incoming sensory signals, has been employed to explain perceptual and cognitive phenomena, and has inspired computational models. There is little understanding, however, of how PC might be implemented at a mechanistic level in individual neurons, particularly within the auditory system. Using natural birdsongs, we examined responses of single neurons (n = 214) in caudomedial nidopallium (NCM), an analog of higher-order auditory cortex, in anesthetized European starlings listening to conspecific songs. We first trained a simple feedforward, single layer, temporal prediction model (TPM; Singer et al., 2018) to predict short segments (10.5 ms) of future birdsongs based on past 170 ms spectrographic samples while varying the number of TPM hidden units (256, 128, 64 and 32 units) to define a "latent" predictive feature space of corresponding dimensionality. To examine the predictive coding of NCM neurons, we modeled the composite receptive field (CRF) of each neuron, using the Maximum Noise Entropy method (MNE; Kozlov et al., 2016, Fitzgerald et al., 2011), in which the stimulus representation was either the short-time Fourier transform spectrogram or the projection of the spectrogram into a TPM latent space . This yielded a version of each neuron's CRF fit to either (1) all features of conspecific song (fft-CRF) or (2) only the predictive features of song (TPM-CRF). As a control, we also computed the CRF for the spectrogram projected into the latent space of an auto-encoder (AVGN; Sainburg et al., 2020) matched in dimensionality to the TPM. The TPM-CRFs yield significantly better predictions of each neuron's empirical spiking response to novel song, accounting for 60.41% of the response variance (mean r = 0.71±0.04), compared to both the fft-CRF ($p < 3.1 \times 10^{-32}$, paired t-test) and the AVGN-CRF ($p < 1.1 \times 10^{-35}$, paired t-test). The AVGN-CRFs also outperformed the spectrogram-based fft-CRFs. Almost all of the observed CRF model improvement can be attributed to the contribution of non-linear CRF components; the linear component of all the CRF models yielded similar response predictions. Thus, the auditory responses of NCM neurons are best modeled collectively by a signal representation that captures covariant structure in the predictive spectrotemporal acoustic features of song. This provides strong support for the notion of a predictive auditory code in songbirds.

Disclosures: S. Rudraraju: None. **B.H. Theilman:** None. **T. Gentner:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC016363

Title: Visual stimuli modulate responses to single and multiple sounds in the macaque inferior colliculus

Authors: *M. N. SCHMEHL, J. M. GROH;
Neurobio., Duke Univ., Durham, NC

Abstract: Visual cues can influence brain regions that are sensitive to auditory space (for review, see Schmehl & Groh, *Annual Review of Vision Science* 2021). However, how such visual signals in auditory structures contribute in the perceptual realm is poorly understood. One particularly interesting possibility is that visual inputs help the brain distinguish among different sounds, allowing better localization of behaviorally relevant sounds in a noisy environment (i.e., the cocktail party phenomenon). Our lab previously reported that when two sounds are present, auditory neurons may switch between encoding each individual sound across time (Caruso *et al*., *Nature Communications* 2018). We sought to study how pairing a light with one of two sounds might change these time-varying responses (e.g., Atilgan *et al*., *Neuron* 2018).
To test this, we trained one rhesus macaque (*Macaca mulatta*, female, 14 years old) to localize one or two sounds in the presence or absence of accompanying lights. While the monkey performed this task, we recorded extracellularly from single neurons in the inferior colliculus (IC), a critical auditory region that receives visual input and has visual and eye movement-related responses. We found that pairing a light with a sound can change an IC neuron's response to that sound, even if the neuron is unresponsive to light alone. Further, when two sounds are present, pairing a light with one of the sounds can change a neuron's time-varying response to the two sounds. Together, these results suggest that the IC alters its sound representation in the presence of visual cues, providing insight into how the brain combines visual and auditory information into a single perceptual object.

Disclosures: M.N. Schmehl: None. **J.M. Groh:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.07

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01DC018621
NSF IOS-1942480

Title: The impact of acoustic environment on noise invariance in the zebra finch auditory cortex

Authors: *S. M. MOSELEY[1], C. D. MELIZA[2];
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Abstract: Vocal communication requires the ability to detect a signal of interest within a background of competing sounds with similar acoustics (the "cocktail-party problem"). This ability has been attributed to neurons that selectively respond to a signal of interest while being robust to increasing levels of background noise. Noise-invariant neurons have been observed in several nonhuman species, but it is not yet known how they develop. We hypothesize that noise-invariance requires early experience in complex acoustic conditions. Zebra finches (*Taeniopygia guttata)* are an excellent model for studying communication in noise to test this hypothesis. Because zebra finches live in large colonies, not only must adults solve the cocktail-party problem, but young fledglings need to isolate their tutor's song from the colony background in order to learn and copy that song into adulthood. We predict that this early exposure to colony noise instructs the development of noise-invariant neurons in the zebra finch pallium. We expect that the effects of the absence of colony noise on noise-invariance can be seen as early as the song learning phase of zebra finch development and that these differences in noise-invariance will persist into adulthood. To test this, we reared birds in either the presence or absence of colony noise. Single-unit recordings were performed across the auditory pallium in both song-learning juveniles and adults. During recording, subjects were presented conspecific stimuli embedded in colony noise at varying signal-to-noise ratios. To quantify noise-invariance, neural responses to original stimuli and stimuli embedded in noise were compared using average firing rates and a linear decoder. Consistent with predictions, average firing rates are more noise-invariant in song-learning juveniles that were raised with colony noise.

Disclosures: S.M. Moseley: None. **C.D. Meliza:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.08

Topic: D.06. Auditory & Vestibular Systems

Support: R01DC018650
R00DC015014
Kavli NDI Distinguished Postdoctoral Fellows Program

Title: A default role of the auditory cortex in goal-directed discrimination learning

Authors: *C. DRIEU[1], Z. ZHU[1], A. WANG[1], K. FULLER[1], S. ELNOZAHY[1], S. OSTOJIC[2], K. KUCHIBHOTLA[1];
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Abstract: Learning how to optimally respond to cues in the environment is crucial for survival. Previously, we showed that goal-directed learning can be dissociated into two behavioral phases: rapid 'acquisition' in which stimulus-action contingencies are learned, and slower 'expression' that gradually reveals the learned content. At the neuronal level, the auditory cortex (AC) is well positioned to play a role in both learning processes as it receives sensory, motor, valence and contextual signals. However, whether and how the AC is involved in both learning processes is unknown. We address this using probabilistic optogenetics and two-photon calcium imaging over learning. We train mice on a pure tone, go/no-go task where mice learn to lick to a tone (S+) and withhold from licking to another (S-); contingency learning is assayed daily using probe trials when reinforcement is unavailable. We optogenetically inactivate the AC on 90% of reinforced trials during learning and measure behavioral performance on probe trials (no inactivation). We observe a robust delay in learning, suggesting a critical role for the AC in contingency acquisition. In separate mice, we test the role of AC in expression by inactivating AC on 50% trials during learning and comparing trials with and without inactivation. Mice discriminate poorly when AC is inactivated early in learning. Surprisingly, this deficit gradually wanes and by the end of learning is only minimally present. These data argue that the AC enables expression but then teaches itself out. How then are these learning phases implemented in the AC? To address this, we use longitudinal, two-photon calcium imaging of the same large population of excitatory neurons in L2/3 of AC over learning (1,050±124 neurons/mouse). We isolate learning-related dynamics by comparing mice learning the task (n=4) to those passively listening to the same tones (n=2). To uncover low-dimensional structure in our high-dimensional data, we use clustering methods and unsupervised low-rank tensor decomposition (TCA). We observe distinct neural clusters whose stimulus selectivity evolve at the rates of acquisition and expression. Interestingly, learning enhances S- representation more prominently than the S+, suggesting a feedforward role in behavioral inhibition. TCA preliminarily reveals a striking reward component in which S+-responsive neurons shift to firing during reward consumption, suggesting a direct role in reward learning. Overall, our work suggests a default but temporary role for the AC in discrimination learning that is mediated by a network that shifts from being largely stimulus-driven to one that is optimized for behavioral needs.

Disclosures: C. Drieu: None. **Z. Zhu:** None. **A. Wang:** None. **K. Fuller:** None. **S. Elnozahy:** None. **S. Ostojic:** None. **K. Kuchibhotla:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.09

Topic: D.06. Auditory & Vestibular Systems

Support: ANR-19-CE37-0016
Fondation Pour l'Audition (FPA RD-2018-6)

Title: Filtering properties of the auditory cortex of mice during attentive listening

Authors: C. BARDIN[1], S. WOLF[1], L. SWALES[1], A. LOURDIANE[1], A. MOLINERI[1], L. BOURDIEU[1], C. VENTALON[1], S. A. SHAMMA[2], Y. BOUBENEC[2], ***J.-F. LEGER**[1];
[1]CNRS - Ecole Normale Superieure, PSL, Paris, France; [2]Lab. des Systemes Perceptifs, Paris, France

Abstract: Auditory attention is a rich and complex process that allows the mind to select specific information. It provides us with the ability to focus only on a fraction of our acoustic environment, as for instance when we attend to a single speaker's voice in a multi-speaker background. It has been shown in ferrets and in mice that attention can modulate the activity in the primary auditory cortex (A1). Sound feature encoding and extraction at this first cortical stage depend not only on the physical nature of the acoustic stimuli, but also on whether listening is performed passively or during an active task. However, although active listening is thought to help select relevant sounds from the noisy background, it is still unclear how A1 modulation leads to a filtering process. Previous studies have shown conflicting observations in A1, with either an increase (Fritz et al. Nat.Neurosci 2003) or decrease (Otazu et al. Nat. Neurosci. 2009) in the selectivity of A1 neurons to sounds on which attention is focused, or a combination of both (Kuchibhotla et al. Nat. Neurosci. 2016). How these different categories of modulation may contribute to a filtering attentional process and ultimately enhance effective perception of target or noisy sounds remains elusive. We address this question by recording the superficial layers of A1 with two-photon microscopy in mice performing either an operant auditory discrimination task or passively listening to the same sounds. We developed an approach to monitor the animal's readiness for the task, using fine movements of body parts extracted with DeepLabCut. This monitoring shows that our expert mice mobilize their attentional resources based on the structure of the protocol that presents active and passive trials in distinct blocks, with only 2-3 trials required to understand any block category transition. Optical recordings reveal strong inhibition in response to the target sound during attentive episodes for the majority of cells in the supragranular layers. However, a minority of neurons remain strongly responsive to the target, increasing the contrast between them and the rest of the silenced cells in A1, and resulting in a potential global filtering effect for the brain structures reading this A1 activity. Further characterization of this minority of cells shows that a fraction is also active when the reward is expected, suggesting a role that goes beyond the strict perception of acoustic information.

Disclosures: C. Bardin: None. **S. Wolf:** None. **L. Swales:** None. **A. Lourdiane:** None. **A. Molineri:** None. **L. Bourdieu:** None. **C. Ventalon:** None. **S.A. Shamma:** None. **Y. Boubenec:** None. **J. Leger:** None.

Poster

P448. Auditory Processing: Perception, Cognition, and Action

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P448.10

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 1R01DC018802

Title: Movement-related activity in auditory cortex changes as mice learn to expect a self-generated sound

Authors: *W. ZHOU, N. AUDETTE, D. M. SCHNEIDER;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: To properly process sensory stimuli generated by one's own movements, the brain may use predictive processing in which copies of movement information are used to modulate sensory responses to self-generated stimuli. In mouse auditory cortex (ACtx), baseline and sound-triggered activity are influenced by movement, including a marked suppression of sound-evoked responses during movement compared to rest. This suppression of sound responses during movement can be shaped by learning to reflect the properties of self-generated sounds. Here, we show that activity in ACtx right before an expected self-generated tone is also modulated by movements and may contain information about the upcoming sound. We developed a simple task in which mice push a lever and hear a predictable self-generated tone. We recorded the activity of neurons in auditory cortex on the first and last days of training with the sound-generating lever. We found that baseline population firing rates in ACtx ramp up several hundreds of milliseconds before the self-generated tone and continue until the sound is heard. Both regular-spiking (RS) and fast-spiking (FS) units are involved in the ramping-up; and the change is much more strongly observed in deeper layers. Comparing the last session of training to the first, we noticed that the increase of pre-tone activity becomes more significant with learning, especially among those units that are responsive to the expected self-generated tone frequency. This change in activity was particularly concentrated in RS neurons. Across animals, the change in pre-tone activity before and after learning was weakly correlated with emergence of predictive suppression during sound playback. Together, these findings suggest that pre-tone activity during movement in ACtx may facilitate the processing of self-generated sounds.

Disclosures: W. Zhou: None. **N. Audette:** None. **D.M. Schneider:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.01

Topic: D.07. Vision

Support: NIH Grant EY009593

Title: Spatial and temporal precision of inhibitory and excitatory neurons in the murine dorsal lateral geniculate nucleus

Authors: A. S. GORIN[1], S. AHN[1], V. SURESH[2], U. M. CIFTCIOGLU[1], Y. SU[3], Y. MIAO[1], F. T. SOMMER[4], J. A. HIRSCH[1];
[1]Neurobio., [2]Biol. Sci., [3]USC, Los Angeles, CA; [4]Univ. California, Helen Wills Neurosci Inst., Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Local inhibitory interneurons within the dorsal lateral geniculate nucleus (dLGN) of the thalamus innervate relay cells and each other to influence every spike sent to cortex. Visual processing in dLGN has been studied mainly in cat, where almost all relay cells have receptive fields made of two concentric subregions, a center and a surround, that have the opposite preference for stimulus polarity. Further, there are push-pull responses within each subregion—e.g. bright stimuli placed in an On subregion excite and dark ones inhibit. Push is supplied by retinal ganglion cells that have the same center sign as the target cell. Because local interneurons in cat also have center-surround receptive fields, we hypothesized that they synapse with relay cells of the opposite center sign to provide pull. It is difficult to target interneurons *in vivo*, however, and study their responses in depth. Moreover, pull is only one example of inhibition in dLGN; computational studies, for instance, suggest that inhibition from local interneurons in cat dLGN improves temporal precision in relay cells. Thus, with the dual goals of learning about intrathalamic inhibition and how this compares across species, we turned to mouse and used optogenetics to target interneurons. Our previous work showed that the largest single class of murine relay cells had center-surround receptive fields with push-pull responses; most other relay cells had various types of overlapping On-Off responses. This diversity suggested that mechanisms and forms of inhibition differ across species. Using spike-triggered analyses, we identified murine interneurons with On, Off, On-Off, and center-surround receptive fields and also found that the range of receptive-field sizes was similar for interneurons and relay cells. Thus, individual interneurons can provide feature-specific inhibition to their targets. Given that relay cells and interneurons provided information over similar spatial scales, we asked if they fired with equivalent temporal precision by calculating the reliability of spike trains across repeated presentations of natural movies shown at different contrasts. Although, a subset of interneurons were as temporally precise as the most reliable relay cells, as a population,

interneurons were less precise. In addition, relay cells fired with increasing reliability as stimulus strength reached the maximum, but this trend was less robust for interneurons and saturated at lower contrasts. Thus, our results suggest inhibition generated within dLGN conveys diverse visual features, varies over a wide range of spatial scales, can be precisely timed, and is sensitive to stimulus contrast.

Disclosures: A.S. Gorin: None. **S. Ahn:** None. **V. Suresh:** None. **U.M. Ciftcioglu:** None. **Y. Su:** None. **Y. Miao:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.02

Topic: D.07. Vision

Support: EY009593
EY024173
GM103436
EY031322

Title: Structure and function relationships of interneurons in the carnivore and rodent lateral geniculate nucleus of the thalamus

Authors: *S. AHN[1], R. MELTZER[1], A. KUMAR[1], A. GORIN[1], D. ALSTON[2], V. SURESH[1], M. A. FOX[3], J. A. HIRSCH[1], M. E. BICKFORD[2];
[1]USC, Los Angeles, CA; [2]Univ. of Louisville, Louisville, KY; [3]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Local interneurons in the dorsal lateral geniculate nucleus (dLGN) of the thalamus influence every spike visual cortex receives; they provide feedforward inhibition to each other and to relay cells. Here, we focused on understanding structure/function relationships in inhibitory circuits in dLGN from a comparative perspective using species with good (carnivore) versus poor (rodent) visual acuity. Our anatomical studies were motivated by whole-cell recordings from thalamic interneurons in vivo. For carnivore interneurons, membrane currents were dominated by serial, unitary inhibitory postsynaptic currents, each of which was preceded by a depolarizing notch. These recordings suggested the presence of triadic circuits in which pairs of interneurons share input from the same retinal afferent and connect with each other via dendrodendritic synapses. By contrast, recordings from rodent interneurons exhibited trains of unitary excitatory postsynaptic currents, indicating different types of connectivity between species. To explore synaptic connections in inhibitory circuits, we first used transmission electron microscopy to analyze single sections of GABA-stained tissue of ferret and mouse. Our

results revealed numerous dendrodendritic synapses between interneurons. To gain a fuller view of synaptic arrangements that include interneurons, we made 3D reconstructions using tissue prepared with serial block-face scanning electron microscopy. We found triads involving two interneurons in both species; however, there was great variation between species. First, there were substantial differences in the composition of synaptic glomeruli, the structures in which triadic circuits usually occur. In carnivore, glomeruli involved a large central retinal bouton surrounded by several interneuronal dendrites. Rodent glomeruli, conversely, comprised only few interneuronal dendrites encircled by small retinal boutons, and the interneurons that participated in triads also received input from multiple retinal terminals. In addition, in carnivore alone, we found interneuronal dendritic segments that did not receive retinal input but formed many connections with other interneuronal dendrites. Further, dendrodendritic synapses between interneurons were evenly distributed between shafts and appendages in carnivore but concentrated on appendages in rodent. These results suggest that there is a more powerful and flexible inhibition in carnivore versus rodent dLGN.

Disclosures: S. Ahn: None. **R. Meltzer:** None. **A. Kumar:** None. **A. Gorin:** None. **D. Alston:** None. **V. Suresh:** None. **M.A. Fox:** None. **J.A. Hirsch:** None. **M.E. Bickford:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.03

Topic: D.07. Vision

Support: NIH 5R01EY009593

Title: Modeling responses of local interneurons in the mouse dorsal lateral geniculate nucleus

Authors: *Y. MIAO[1], A. GORIN[1], S. AHN[1], F. T. SOMMER[2], J. A. HIRSCH[1];
[1]USC, Los Angeles, CA; [2]Univ. California, Helen Wills Neurosci Inst., Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Visual information from retina is processed by neural circuits in the dorsal lateral geniculate nucleus (LGN) of the thalamus prior to reaching cortex. Almost all relay cells in dLGN of highly visual animals have receptive fields with a stereotyped profile--a center-surround arrangement of On and Off subregions that have push-pull responses to stimuli of the opposite contrast (i.e., where a bright stimulus excites, a dark one inhibits and vice versa). This is the most common receptive field structure for relay cells in the rodent geniculate as well (~ 40%). The remaining relay cells have variations of On-Off responses that are sometimes sensitive to stimulus direction or orientation, properties that inhibition appears to sharpen. We asked how local interneurons might contribute to push-pull and other forms of inhibition in

mouse. To address this issue, we developed a preparation that allows us to identify interneurons optogenetically during multielectrode recordings. We tested competing hypotheses about whether or not interneurons provide a one-size-fits-all form of inhibition to relay cells, as is largely the case in mouse cortex, or if the inhibition they provide is feature specific. Our results lend support for the latter idea--interneurons had diverse types of receptive fields, including center-surround, On, Off, On-Off varieties.
Our ultimate aim is to learn how interneurons influence the spike trains relay cells transmit to cortex. Toward this goal, we have begun to build computational models that predict responses of each type of interneuron we sampled. This step is necessary because it is impossible to optimize stimuli (e.g., size, contrast) for every cell, given that recording times are limited and multiple units are mapped at once. Thus, we used a generalized linear model framework to fit changes in firing rate of optotagged interneurons elicited by sparse-noise stimuli (bright and dark squares flashed separately at randomized positions in the stimulus grid), using two linear components in the case of On-Off cells. These models performed well, achieving values for explained variance of 70% or above. Our next step will be to build integrate-and-fire models of murine relay cells. Then, we will transform the interneurons' spike trains to synaptic conductances and combine these with the integrate-and-fire models to explore how inputs from different types of local interneurons might generate the patterns of visually-evoked inhibition we have observed during whole-cell recordings from relay cells.

Disclosures: Y. Miao: None. **A. Gorin:** None. **S. Ahn:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.04

Topic: D.07. Vision

Support: NIMH Grant MH002964
NIMH IRP Rodent Behavioral Core, Grant MH002952

Title: The mouse vLGN circuit organization underlying its role in visual behaviors

Authors: *C. BEIER, M. THURMAN, M. THOMSEN, R. KOMAL, S. HATTAR;
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Abstract: Light perception is parsed into retinal pathways that drive image-forming vision (pattern vision, gaze control) or directly affect light-influenced behaviors (circadian photoentrainment and the effects of light on sleep, feeding, pupil size and mood). Retinal ganglion cells (RGCs) convey visual features such as color or motion to targets in the brain.

Light-influenced behaviors are driven by a subset of RGCs, the intrinsically photosensitive RGCs (ipRGCs). Understanding visual feature integration or segregation in the brain is key to understanding how mammals respond to their environment. The mouse ventral lateral geniculate nucleus (vLGN) provides a unique opportunity to investigate this, as it is the only brain region to receive both convergent (rostral vLGN) and divergent (caudal vLGN) innervation from image-forming RGCs and ipRGCs. Here, we investigate the circuit organization and function of the mouse vLGN.We mapped efferents and afferents of vLGN *Penk*^{Cre} and *Nos1*^{Cre} cell types with Cre-dependent anterograde viruses and monosynaptic rabies G-deleted virus. Chemogenetic manipulation, using Tetanus toxin (TenT) or excitatory DREADD with CNO to silence or activate cells, revealed the role of vLGN *Penk*^{Cre} cells in modulating pupil size. We then investigated *c-Fos* induction within the vLGN following circadian phase shifting light pulses.The rostral vLGN does not show the same cellular stratification as the caudal vLGN. *Penk*^{Cre} cells send projections to the suprachiasmatic nucleus (SCN) and the olivary pretectal nucleus. *Nos1*^{Cre} send projections to the SCN. *Penk*^{Cre} cells, but not *Nos1*^{Cre} cells, in the caudal vLGN are post-synaptic to ipRGCs. *c-Fos* induction in the caudal vLGN increases in *Penk*^{Cre} cells in response to a phase advancing light pulse but shows minimal change to a phase delaying light pulse compared to dark controls. Silencing/activating vLGN *Penk*^{Cre} cells causes an increase/decrease in pupil constriction during a light step.We find that circuit organization in the vLGN changes along the rostral-caudal axis and reflects retinal innervation to the vLGN. Not only do *Penk*^{Cre} cells modulate pupil size, but our data suggests they may modulate phase advancements in response to a light pulse. *Nos1*^{Cre} cells, which stratify with image-forming RGC inputs, also send projections to the SCN and could play an unexpected role in circadian photoentrainment. Taken together, this suggests the caudal vLGN circuit organization maintains visual feature segregation to modulate pupil size but integrates visual features to modulate circadian photoentrainment.

Disclosures: C. Beier: None. **M. Thurman:** None. **M. Thomsen:** None. **R. Komal:** None. **S. Hattar:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.05

Topic: D.07. Vision

Support: NIH NS109978
NIH NS107968
NIH T32NS096050

Title: Distinct contributions of arousal and movement to visual responses in dLGN

Authors: *K. PEELMAN[1], A. DEL CASTILLO VALERIO[2], B. HAIDER[1];
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Abstract: Neural responses to sensory inputs depend on behavioral factors such as changes in arousal, movement, and cognition. These effects have been measured throughout cortex but less so in thalamus. Studies of head-fixed mice agree that locomotion strongly affects responses in visual cortex, but effects in lateral geniculate (dLGN) remain unclear; some studies suggest no impact of locomotion, while others suggest increased baseline and evoked activity, with broadening of visual spatial selectivity. Locomotion partly exerts these effects by increasing arousal, as inferred by measuring pupil dilation. It remains unclear how arousal impacts visual responses in dLGN in the absence of locomotion, and if this affects spatial selectivity. We addressed these questions by recording continuous changes in pupil size and periorbital facial movements in awake, head-fixed, stationary mice (n = 6), while simultaneously recording dLGN neurons (n=288) with high-density NeuroNexus and Neuropixels probes. We used pupil area to distinguish high and low arousal levels, and distinguished high and low motion energy due to spontaneous movements of the face and eye. We presented visual stimuli (7° squares, 0.1s duration) spanning > 90° of one visual hemifield, and examined spatiotemporal responses in the receptive field (RF) as a function of pre-stimulus arousal and motion energy levels. We found that arousal level shaped dLGN responses more strongly than movement. Baseline firing was significantly greater with higher versus lower arousal ($p<0.05$). These effects were not simply reflective of movement: baseline firing was similar across trials with low versus high motion energy ($p = 0.10$). However, visually evoked activity was significantly reduced at high arousal ($p<0.05$), and these effects were also not entirely due to movement: evoked firing was similar for low versus high motion energy ($p = 0.80$). Finally, we examined effects on spatial selectivity by measuring changes to RF width. RF widths were preserved across arousal levels (9.7° vs 9.2° ; $p=0.20$), despite significant changes to spontaneous and evoked firing. However, RF widths were significantly greater (9.8° vs 8.9°; $p<0.05$) during trials with high versus low levels of movement, and this broader spatial selectivity was most apparent on trials with high arousal ($p<0.05$). Our preliminary results suggest that in stationary mice, arousal level modulates spontaneous and visually evoked activity of dLGN neurons with minimal effects on spatial selectivity. These effects are not simply explained by movement levels. Movement does appear to broaden spatial selectivity, but only when arousal is also elevated.

Disclosures: K. Peelman: None. **A. Del Castillo Valerio:** None. **B. Haider:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.06

Topic: D.07. Vision

Support: NIH Grant EY027888
William M. Woof Foundation

Title: Correlating extracellular spike shapes and neuronal responses in the lateral geniculate nucleus of awake macaques

Authors: *S. SUN[1], J. S. PEZARIS[2];
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Abstract: Have we identified all cell types in the lateral geniculate nucleus of the thalamus (LGN)? Unlike the exhaustive determination of the retina, key populations in LGN may have been missed through sampling bias. Of retinal cells projecting to LGN, 80% carry signals associated with classic thalamic responses. We want to identify the missing 20% through rigorous analysis of LGN recordings in awake monkeys.

Extracellular spikes (or action potentials) are instrumental in studying neuronal responses. Spikes are dominated by brief negative-voltage excursions when measured with traditional extracellular metal electrodes, caused by the action of sodium and potassium channels. Spike waveforms have been used to classify the visual pathway cells into either regular-spiking or fast-spiking units, commonly associated with excitatory and inhibitory neurons, respectively. With the development of dense multi-electrode arrays (MEA) and sophisticated spike-sorting algorithms we can now sample the brain, and in particular the LGN, with detailed identification of spike waveforms and reduced sampling bias to reveal previously overlooked signals.

Using these tools, we attempt to characterise the full range of neuronal responses in the LGN of awake monkeys. We find three distinct classes of spike waveforms: negative-dominant waveforms (55%) consistent with what is commonly reported as regular- or fast-spiking or broad- and narrow- spiking; triphasic waveforms (23%) that are like negative-dominant waveforms but have an initial positive peak; and positive-dominant waveforms (22%) that are not often reported in the literature. We correlate these spike classes against the response characteristics of corresponding cells to visual mapping stimuli to identify relationships between spike shape and neuronal class (magnocellular, parvocellular, koniocellular or, importantly, other). A little more than half of the cells (58%) had their receptive field (RF) successfully mapped. Out of the remaining non-mapped LGN cells, 89% responded (with statistical significance) to the mapping stimulus versus the immediately preceding uniform screen. This observation indicates that, due to the sampling biases inherent with traditional electrodes, the population of LGN cells may be broader than what we originally thought as standard. The ongoing effort will answer the question of what spike shapes and visual responses characterise the missing 20% of LGN cell types.

Disclosures: S. Sun: None. **J.S. Pezaris:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.07

Topic: D.07. Vision

Support: R01-EY023581
P30-EY003039
R01-EY023603

Title: Spike encoding of increment sensitivity in macaque retinal ganglion cells and LGN neurons

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Abstract: Retinal ganglion cells (RGCs) and neurons in the lateral geniculate nucleus (LGN) shape the visual signals conveyed from the retina to the brain. At the perceptual level, the intensity response function of individual cone photoreceptors varies between cones and depends on distance from the fovea (Harmening et al. 2014). As a result, we wondered if psychometric thresholds from single cones are limited by RGC or LGN spiking responses, which may be seen by computing comparable neurometric functions. We analyzed optic tract and LGN extracellular recordings made during receptive field center stimulation in response to increasing increment contrast steps (Sincich et al. 2007). Fourteen ON cells were analyzed: 7 parasol, 2 midget, 2 parvocellular, and 3 magnocellular. We examined 3 spike coding techniques as a function of time over each stimulus trial: spike count, summed instantaneous spike frequency (ISF), and summed probability of LGN spiking given an RGC spike train (P(spike)). These data were fit with 3 neurometric functions: Michaelis-Menten, exponential, and logistic. Estimated threshold values were defined as the half-height response in the fits. Response suppression at high contrasts was evident in 6 parasol cells; fitting for these was done only on the rising arm of the data. Three parasol cells were excluded because sampling at low contrasts was insufficient to produce useful fits. Other cells did not show suppression at high contrasts. The logistic function fit the summed P(spike) data better than the other two fitting functions; this was true for all RGC cells. For most cells, summed ISF fits resulted in the highest threshold estimates compared to the fits for spike count or summed P(spike), while fits of spike count tended to result in lower thresholds. RGC threshold values were lowest for parasol cells, averaging ~0.5 log units below midget cells. In contrast, the average difference between magnocellular and parvocellular neurons was negligible; however stimuli were not luminance matched for these cells. Parasol cells had steeper neurometric slopes than midget cells, for all fit types. Parvocellular and magnocellular neurons had similar slopes regardless of fit type. These exploratory analyses show more diversity in the neurometric function between RGC cell classes than LGN cell classes, suggesting sensitivity to contrast will be more distinct at the retinal level than the thalamic level. Future work will examine how these analyses may be used for responses to single cone targeted

stimulation, and help set contrast ranges for experiments that may identify the relationship between neurometric functions and human psychometric performance.

Disclosures: K.M. Ramsey: None. **L.C. Sincich:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.08

Topic: D.07. Vision

Support: NIH/NEI 1R01EY028266

Title: Identifying the layers in the human LGN using quantitative and functional MRI

Authors: *I. YILDIRIM[1], K. HEKMATYAR[2], K. A. SCHNEIDER[1,2];
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Abstract: Identifying the magnocellular (M) and parvocellular (P) parts of human lateral geniculate nucleus (LGN) has been challenging yet remains an important goal because of the different functional properties of these pathways in the brain. Previous research used the magnitude of responses to different types of stimuli that bias M and P regions (e.g. Denison et al., 2014). However, a problem with this functional separation has been raised due to the hilum of LGN showing higher response amplitudes (DeSimone & Schneider, 2019), confounding the responses to tailored stimuli. To resolve the discrepancy in the literature, we have employed three methods that do not depend on stimuli-tuned responses to anatomically and functionally segment the LGN layers. **Method:** Three participants were scanned with a 3T Siemens Prisma magnetic resonance imaging (MRI) scanner in multiple days. We used a quantitative method (qMRI) measuring T1 relaxation time (qT1) as well as two functional MRI (fMRI) experiments to measure the eye dominance signals in LGN. For the qMRI scans, an MP2RAGE sequence (0.7 mm isotropic, TR = 5 s, TE = 3.6 ms, TI_1/TI_2 = 0.9/2.75 s, α_1/α_2 = 3/5°) was used to calculate qT1 maps. By averaging 16 qT1 maps of each subject, we outlined the six LGNs. For the fMRI scans (multiband sequence, 1.5 mm isotropic, TR = 1.5 s, TE = 39 ms), two visual tasks were used in separate days in ten 5-minute sessions. In one task, subjects viewed a checkboard flickering at 4Hz while alternately opening one eye and closing the other for 15 s. In the second task, both eyes remained open but a grating rotating at 1 cycle/s was shown alternating between eyes using a polarizing filter synchronized to a ProPixx projector and polarized glasses. **Results:** By fitting the qT1 maps in each LGN to a 2-component Gaussian mixture model, we separated the M and P regions. These regions were in line with the anatomical expectations; the dorsolateral P voxels had shorter T1 relaxation than M voxels, suggesting more myelination in

the P layers. With the fMRI data, we aimed to identify the contralateral eye layers of the M and P as these are distant in LGN. The categorization of the eye dominance was correlated but not entirely consistent between the two tasks. The organization of the contralateral layers were more accurate with the task having one eye closed at a time; nevertheless, the contralateral M layer did not appear reliably, probably due to this layer being thin and most ventral. **Conclusion:** These results indicate that functional segregation of the LGN remains challenging. We suggest that using quantitative methods are more promising to identify M and P layers and multiple methods should be used to reliably segregate the layers functionally.

Disclosures: I. Yildirim: None. **K. Hekmatyar:** None. **K.A. Schneider:** None.

Poster

P460. Subcortical Visual Pathways III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P460.09

Topic: D.07. Vision

Support: NIH NEI EY031052
NIH NEI EY025219

Title: Excitotoxic lesions in the ferret retina differentially impact X and Y dorsal lateral geniculate neurons

Authors: *J. YANG[1], K. R. HUXLIN[2], F. BRIGGS[1];
[1]Dept. of Neurosci., [2]Flaum Eye Inst., Univ. of Rochester, Rochester, NY

Abstract: The dorsal lateral geniculate nucleus (dLGN) of the thalamus relays the majority of inputs from the eyes to the brain. It contains physiologically distinct neuronal types that make up parallel processing streams for incoming visual information. Although much is known about the functional properties of dLGN neurons in the intact visual system, surprisingly little is known about neurophysiological changes that may occur after removal of retinal input, as may occur in retinal disease. Here, we address two significant, open questions: 1) what happens neurophysiologically when the dLGN is deprived if its retinal input; and 2) does loss of retinal input differentially impact dLGN neurons in the parallel streams? We induced excitotoxic lesions of the retina in ferrets by injecting kainic acid (KA) into one eye. We verified retinal ganglion cell (RGC) loss in the lesioned eye with electroretinography and Optical Coherence Tomography. We measured visually evoked potentials, the thickness of the retinal nerve fiber layer, and counted fluorescently-tagged RGCs. We also recorded from dLGN neurons contralateral and ipsilateral to the KA-injected eye using multi-electrode arrays and measured their responses to a variety of visual stimuli including drifting sinusoidal gratings and white noise m-sequences. Our preliminary data suggest that RGCs with large somas are preserved

compared to those with small somas in the injected ferret retinas. Correspondingly, we observed normal transient responses but altered sustained responses among dLGN neurons recorded contralateral to the lesioned eyes. Some dLGN neurons contralateral to the lesioned eye exhibited spatial receptive fields that reflected enhanced cortical input. Together, these findings show that the Y retinogeniculate pathway may be less susceptible to degeneration compared to the X pathway. The alteration in spatial receptive field structure also suggests that dLGN neurophysiology may shift to reflect intact corticogeniculate input. Together, our findings reveal that retinal lesions produce changes in both the temporal and spatial receptive fields of dLGN neurons, although these effects may vary across the parallel streams.

Disclosures: J. Yang: None. **K.R. Huxlin:** None. **F. Briggs:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.01

Topic: D.07. Vision

Support: NIH R01EY026025

Title: What is a face (neuron)? A psychophysics study of synthetic preferred stimuli for face-selective neurons

Authors: A. BARDON[1], *W. XIAO[2], C. R. PONCE[3], M. S. LIVINGSTONE[4], G. KREIMAN[2]; [1]Caltech, Pasadena, CA; [2]Harvard Univ., Boston, MA; [3]Neurosci., Washington Univ. At St. Louis, Saint Louis, MO; [4]Harvard Med. Sch., Boston, MA

Abstract: In the primate, inferior temporal cortex (IT) is known to contain neurons that show generally stronger activity in response to images of faces compared to images of other objects. These so-called ‘face neurons’ have been studied extensively, but there remain questions about how well this designation captures their functional properties. Ideally, a face neuron’s responses should align with the subjective perception of a face. Yet, face neurons also respond to clocks, fruits, single eyes, and other objects that share features visually related to but dissociable from faces. To define the functional properties of face neurons more thoroughly, we used a recently described algorithm, XDream (Ponce, Xiao et al. 2019), to synthesize stimuli that strongly activated classically defined face-selective neurons in macaque monkeys; we did the same with non face-selective neurons. A key advantage of XDream is that it can create stimuli not limited to realistic pictures or object categories. We then asked human participants to assess the content of the synthetic images and natural photos (face and non-face objects including cars, fruit, etc.), with particular focus on how much images were perceived as being face-like. We found that while subjects perceived animal features in synthetic images, they generally did not perceive

synthetic images as particularly face-like. Nevertheless, synthetic images tailored to face-selective neurons were perceived as more face-like than control non-face photos and than synthetic stimuli tailored to non face-selective neurons. Moreover, among natural images, there was significant correlation between the subjects' 'faceness' ratings and face-neuron responses to images, even only among non-face images. However, this correlation was reduced when including synthetic images, which received relatively low faceness ratings yet evoked some of the highest firing rates. In summary, we discovered novel stimuli that evoked high firing from face neurons, are perceived as somewhat face-like by humans, yet are clearly not faces. This shows that face neurons are not as categorically tuned as was previously hypothesized. These results are compatible with other recent work on IT, suggesting that IT may be better described as tuned to visual features rather than semantic or perceptual categories.

Disclosures: A. Bardon: None. **W. Xiao:** None. **C.R. Ponce:** None. **M.S. Livingstone:** None. **G. Kreiman:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.02

Topic: D.07. Vision

Title: Towards developing a nonhuman primate model to dissect the neural mechanisms of atypical facial emotion processing in autism spectrum disorder

Authors: *K. KAR;
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Abstract: Recognizing others' moods, emotions, and intent from facial expressions lies at the core of human interpersonal communication and social engagement. This relatively automatic, visuocognitive feature that neurotypically developed human adults take for granted show significant differences in children and adults with autism spectrum disorder (Adolphs et al. 2001). Rhesus macaques are one of the most appropriate animal models to investigate these vision-based, social behaviors because 1) macaques' ability to perform human-like facial emotion recognition 2) our ability to perform targeted fine-grained neural measurements and perturbations in macaques 3) the relevance and translational value of the experimental inferences given the established brain homologies between humans and macaques. Furthermore, we can map the sensory representations across the macaque visual pathways onto specific parts of current artificial neural network (ANN) models of primate vision (Yamins et al., 2014; Kar et al., 2019) to generate specific neural circuit level hypotheses. Here, first I have used macaque brain-tissue mapped artificial neural network (ANN) models of primate ventral stream as a neural circuit level hypotheses generation tool. Interestingly, the ANNs' image-level behavioral patterns

better matched the neurotypical subjects' behavior than those measured in the ASD population (stimuli and task adopted from the Wang and Adolphs, 2017 study). This behavioral mismatch was most remarkable when the ANN behavior was decoded from units corresponding to the primate inferior temporal (IT) cortex. ANN-IT responses also explained a significant fraction of the image-level behavioral predictivity associated with neural activity in the human amygdala (Wang et al. 2017), strongly suggesting that the previously reported facial emotion intensity encodes in the human amygdala could be primarily driven by projections from the IT cortex. Second, preliminary results from both neural recordings in the macaque IT cortex and corresponding behavioral measurements are consistent with these ANN predictions. Taken together, these results support the use of an ANN-guided approach towards developing an NHP model to investigate the neural markers of atypical facial emotion processing in ASD.

Disclosures: K. Kar: None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.03

Topic: D.07. Vision

Support: Packard Fellowship #2020-71377

Title: Is that...a monkey? Macaques detect conspecific features in images synthesized by ventral stream neurons.

Authors: *K. MUELLER[1,2], M. BURKEMPER[1], C. R. PONCE[1,2];
[1]Neurosci., Washington Univ. in St. Louis, St. Louis, MO; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Prototype theory suggests we categorize objects by comparing them to an abstracted average, an internal “prototype” of examples we have experienced (Rosch, 1973; Leopold et al., 2001). If true, this suggests neurons may encode abstractions of key visual attributes in the natural world. We recently showed images can be synthesized under the guidance of neurons from the macaque ventral stream using naturalistic image generators and search algorithms (Ponce, Xiao et al., 2019). When applied to neurons in inferotemporal cortex (IT), this approach often generated images with visual attributes present in pictures of monkeys, as confirmed by neural networks and human visual judgements (Bardon et al. and Kreiman, in prep.). However, it is unknown if monkeys find any similarity between these response-optimized images (termed “prototypes”) and images of conspecifics. Do monkeys find some prototypes are monkey-like and, more fundamentally, can they abstract visual features from photographs? To pursue these questions, we implanted two macaques (A and B) with chronic microelectrode arrays in areas

V1, V4, and IT and synthesized prototypes from single- and multiunit activity. The animals were then trained in a forced decision task in which a pair of images was presented - one sampled from a target category (e.g., monkeys) and another from a distractor category (e.g., wheels, flowers). Making a saccade to the target image resulted in a large reward; distractor choices triggered a time-out. Training photographs were recreated using the generator to control for visual style. When subject performance surpassed 75%, probe trials were introduced and randomly rewarded. Probe image pairs included a neuronal prototype and a random generator image. When trained with conspecifics as the target category, monkeys selected IT prototypes over random generator images more often (A: 59%, SD = 2.5%; B: 78%, SD = 15.7%) and selected V1 prototypes less often (A: 43%, SD = 8.1%; B: 49%, SD = 9.7%). This suggests higher perceived similarity between monkey images and IT prototypes. Next, to validate task design and explore their ability to abstract image features, we trained the animals on geometry-based categories. Targets included scenes with triangular objects (e.g., road signs, hats, cones) and distractors included scenes with round objects (e.g., wheels, coins, balls). In probe trials following training, monkeys were more likely to choose artificial, solid-color triangles over solid-color circles (A: 78%, SD = 0.9%; B: 78%, SD = 0.5%). Together, these results suggest monkeys can abstract visual concepts from the natural world, and neurons of the ventral stream likely encode these concepts.

Disclosures: K. Mueller: None. **M. Burkemper:** None. **C.R. Ponce:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.04

Topic: D.07. Vision

Support: Packard Fellowship #2020-71377

Title: The macaque ventral stream shows a hierarchical structure for animal-feature encoding

Authors: *Z. ZHANG[1], T. S. HARTMANN[2,3], M. S. LIVINGSTONE[2], R. T. BORN[2], C. R. PONCE[1,2];
[1]Neurosci., Washington Univ. in St. Louis, St. Louis, MO; [2]Neurobio., Harvard Med. Sch., Boston, MA; [3]Perceptive Automata, Boston, MA

Abstract: Are there organizing principles for information encoding along the primate ventral stream? Neurons in each visual area are often tested with different types of stimuli, ranging from simple (e.g., lines in V1) to complex (faces in inferotemporal cortex, IT); however, this strategy limits functional comparisons across areas. By comparing neuronal responses to a given stimulus set across areas, we set out to identify brain-wide organizing principles and determine if these

principles are shared by learning-based models of the ventral stream (convolutional neural networks, CNNs).
We measured the responses of neurons in V1, V4, and IT to large natural scenes (N = 6 monkeys, ~448 distinct cortical sites). We mapped responses in non-overlapping regions within each scene, interpolating these responses to every pixel — a technique inspired by weight-sharing convolutional operations of CNNs and applicable to the brain (Arcaro *et al.*, 2020). The resulting "feature maps" served as a common metric across brain regions and CNN layers. To interpret these maps, we (1) used the automated pipeline of DeepLab (Chen *et al.*, 2017) to segment objects within each scene and (2) recruited human annotators to segment each scene using labels independently assigned to it by Google Cloud Vision. Using these segmentations as masks, we tested whether neurons and hidden units in CNNs responded higher inside the masks than outside of them. We quantified this response difference using an area-under-the-curve-based statistic (AUC). As a control, we shuffled the response regions for each scene.
We found these segmentation masks predicted a hierarchy of responses across the ventral stream. Masks identified regions with increased neuronal activity for IT better than they did for V4, and for V4 better than for V1 (AUC mean ± SEM; IT: 1.09 ± 0.04; V4: 0.77 ± 0.03; V1: 0.43 ± 0.02). Notably, semantic masks isolated V1 responses better than they did for shuffled controls (AUC for shuffled maps, across areas: -0.02 ± 0.02 to 0.00 ± 0.01). Generally, CNNs did not show the same hierarchical relationship as the ventral stream, although CNNs with better BrainScore rankings (Schrimpf *et al.*, 2020) showed trends towards this pattern. Finally, classic saliency algorithms (GBVS, Harel *et al.*, 2006) produced maps that correlated best with IT feature maps (Pearson correlation; IT: 0.38; V4: 0.22; V1: 0.20).
Together, these results suggest the monkey ventral stream is partially organized to encode salient background-isolated objects, particularly those containing animal features —even within V1. We conclude that CNNs trained to prioritize animal-like representations may serve as better models of the ventral stream.

Disclosures: Z. Zhang: None. **T.S. Hartmann:** None. **M.S. Livingstone:** None. **R.T. Born:** None. **C.R. Ponce:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.05

Topic: D.07. Vision

Support: the David and Lucille Packard Fellowship #2020-71377
the McDonnell Center for Systems Neuroscience (pre-doctoral fellowship to B.W.)

Seed grant from the Center for Brains, Minds and Machines (CBMM; funded by NSF STC award CCF-1231216)

Title: Climb high and gaze far: mapping tuning landscapes of the ventral stream via image optimization and manipulation

Authors: *B. WANG[1,2], C. R. PONCE[1,2];
[1]Neurosci. Dept., Washington Univ. In St. Louis, Saint Louis, MO; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Vision scientists often characterize neuronal function using tuning curves, defined as neuronal response as a function of a continuous variable. These variables are chosen *a priori* and often control geometric shape transformations (e.g., curvature). Tuning curves have been difficult to define in naturalistic image spaces, partly due to these spaces' high dimensionality and a lack of consensus on parameters. To study neurons in naturalistic image spaces, we used an image manifold parametrized by a deep generative network. Using an evolutionary algorithm (CMAES), we let each neuron "choose" its own image domain of interest via neuron-guided image search. Once a highly activating image evolved, we these experiments on two monkeys, driven by neurons recorded from V1/V2, V4, and IT. Using pre-trained CNNs as models of the visual hierarchy, we also conducted the same experiments in silico.
We found driving neurons across the ventral stream exhibited smooth, bell-shaped tuning peaks on the manifold, reminiscent of classical tuning curves. These peaks were wider and taller than those measured in classic spaces like orientation and curvature. When comparing the neuronal tuning along the ventral stream (from V1 to IT), we found tuning functions became sharper (per Kent function parameter κ, mean±SEM, V1, 0.7±0.1; V4, 1.8±0.2; IT, 3.2±0.3), image search converged more slowly (V1, 12±1 generations; V4, 21±3; IT, 29±2), and generator dimensionality required to achieve high activation became larger. We found these patterns also emerged in CNNs. These findings suggest that neurons in higher visual cortices encode combinations of visual attributes that are statistically rarer and more specific than those in early visual cortex. Finally, we developed data-driven CNN models to identify highly activating features contained within each evolved image. Using tensor factorization, these models can be interpreted as a spatial combination of filters encoding simpler patterns. The models reproduced the neuronal image search process *in silico*, predicted responses to other images and generated cleaner images that also activated the neuron highly. We found IT neurons needed a larger number of filters to model accurately (V1, 3.1±0.4; V4, 4.8±0.6; IT, 6.4±0.5), consistent with their more complex selectivity.
Together, these findings suggest that neurons in the ventral stream are well-designed to encode for image transformations spanning nearly all of image space. Our analysis pipeline extends classic approaches in vision with a novel paradigm that informs us of neuronal tuning in the natural world.

Disclosures: B. Wang: None. **C.R. Ponce:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.06

Topic: D.07. Vision

Support: Packard Fellowship #2020-71377

Title: As simple as possible, but not simpler: measuring the complexity of neural representations and their relationship to natural scenes

Authors: O. ROSE[1,3], J. K. JOHNSON[1], B. WANG[2,3], ***C. R. PONCE**[1,3];
[1]Neurosci., [2]Washington Univ. in St. Louis, St. Louis, MO; [3]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Fast and accurate visual object recognition is key to survival, allowing animals to identify food, predators, and conspecifics in their environment. Early theories of efficient coding suggested the visual system compresses the world by learning to represent features where "information was concentrated" (Attneave, 1954) such as contours, and emphasized that these features would be relevant to behavior (Barlow, 1961). These early views were validated by the discovery that neurons in posterior visual cortex respond to edges and curvature. Still, it remains unclear what other information-rich features are encoded by neurons in more anterior cortical regions such as inferotemporal cortex (IT). Here, we used a generative deep neural network to synthesize images guided by neuronal responses from across the visuocortical hierarchy (neurons at the border of V1/V2, V4 and IT). We refer to these images as "prototypes," as they are hypothesized to represent abstracted features of the visual world. We found IT neurons took longer to develop their prototypes compared to neurons in V1/V2 and V4, as expected if IT encodes more specific patterns. Prototypes varied across areas: V1/V2 prototypes frequently contained achromatic edges, while V4 and IT prototypes contained higher curvatures quantitatively associated with specific visual attributes present in faces, bodies, and fruits. Per measures of complexity such as minimal description length, prototypes were more complex than classic artificial stimulus sets, but less complex than images created spontaneously by the generator or encoded by neural networks. Additionally, prototypes resembled salient visual features and semantic content of natural images, as indicated by the animals' eye movements. Overall, these results suggest prototypes are learned information-rich features akin to contours, supporting the view that the neural code for object recognition is based on compressed features of behavioral relevance.

Disclosures: O. Rose: None. **J.K. Johnson:** None. **B. Wang:** None. **C.R. Ponce:** None.

Poster

P485. Representation of Faces and Bodies I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P485.07

Topic: D.07. Vision

Support: NIH T32 EY013360
Packard Fellowship #2020-71377

Title: Social information shapes intrinsic visual representations in ventrolateral prefrontal cortex

Authors: *O. ROSE[1,2], C. R. PONCE[1,2];
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Abstract: Prefrontal cortex (PFC) is thought to use sensory input to determine behavioral responses, with studies reporting flexible neuronal activity that tracks task demands. This flexibility is called adaptive coding (Duncan, 2001), and suggests PFC gains functional properties needed to solve a given task. However, studies investigating PFC function during passive fixation report maps of sensory organization (Wilson et al, 1993; Romanski & Goldman-Rakic, 2002; Haile et al, 2019). This raises questions: do individual PFC neurons have inherent sensory tuning? Does task learning induce sensory tuning in non-selective neurons or change existing selectivity in tuned neurons? Or do task demands recruit neurons with suitable selectivity? Here we characterized visual response properties of ventrolateral PFC (vlPFC) neurons in male macaques (C, D) trained only on passive fixation. We implanted 32-channel chronic microelectrode arrays, allowing for stable longitudinal recordings from the same cortical sites. We characterized inherent visual properties of these sites, mapping receptive fields (RFs) and selectivity with photographs and artificial images. We found a small subset of vlPFC sites with reliable RFs in both subjects, showing eccentricity (median ± SE) of 17.40° ± 0.11 (monkey C) and 11.43° ± 0.03 (monkey D). Additionally, up to 28% of the 32 vlPFC sites (C: 2.00 ± 0.02 sites; D: 6.00 ± 0.08) showed visual selectivity for individual images; this selectivity was best evoked by images of conspecifics, and could even be measured in sites without a classical RF. Selectivity was then assessed using ethological categories (e.g. macaque fear grin and perinea, fruit, human faces). This selectivity was linked to reward, with some vlPFC neurons showing significantly reduced reward responses after subjects viewed images of perinea or open-mouth threats. Up to 53% of vlPFC sites (n = 32) were selective following image presentation until reward delivery (median ± SE, C: 6.5 ± 0.05 sites; D: 2.5 ± 0.03), and up to 34% were selective following reward delivery (C: 4.5 ± 0.03 sites; D: 2.5 ± 0.02). We then used generative networks to synthesize highly activating images (“prototypes”) under guidance from these neurons (as in Ponce, Xiao et al., 2019). Prototypes contained visual attributes highly reminiscent of conspecifics, such as fur colors/textures, face- and body-like shapes. These results suggest many vlPFC sites have intrinsic visual tuning for conspecific social information. We are investigating if/how task learning changes inherent selectivity of these vlPFC sites, with the goal of characterizing acquired sensory coding schemes used by vlPFC neurons.

Disclosures: O. Rose: None. **C.R. Ponce:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.01

Topic: E.05. Brain-Machine Interface

Title: A neurosurgical tool to record stereoscopic local field potentials

Authors: *J. SEYMOUR[1], A. ABREGO MANCILLA[1], W. KHAN[1], C. WRIGHT[2], M. GHAJAR[1], N. TANDON[3];
[1]Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [2]Rice Univ., Houston, TX; [3]Neurosurg., Univ. of Texas Med. Sch. at Houston, Houston, TX

Abstract: A variety of electrophysiology tools are available to the neurosurgeon for diagnosis, functional therapy, and neural prosthetics. However, no tool can currently address these three critical recording needs: (i) a surgical method that can reach any cortical region in a minimally invasive manner; (ii) record microscale, mesoscale, and macroscale resolutions simultaneously; and (iii) record from multiple brain regions. We present a novel device whose form is based on the stereo-EEG (sEEG) and the physics describing our technique which we refer to as stereo-LFP (sLFP). sLFP quasi-static EM modeling predicted improved signal-to-noise and directional sensitivity when recording as far as 4mm from a neural source. In our model, we also compare the amplification and sensitivity of microelectrodes (varying dimensions) to state-of-the-art directional leads now commercially available. To validate this model with *in vivo* data, we implanted a novel 128-channel array, sLFP array, into the rat barrel cortex and stimulated 9 whiskers. sLFP was manufactured using polyimide-based microelectrodes wrapped onto a stainless-steel stylet for an outer diameter of 610-μm (Fig.1A). A small burr hole was made above the barrel cortex where a tetrode is first implanted, and 9 whiskers are stimulated (Fig.1B). sLFP was next implanted in the same location. Our results showed agreement with the EM predictions regarding amplitude and SNR improvement relative to microwires. To test our ability to measure distant sources, we used a linear discriminant analysis model to predict which whisker was stimulated. An example confusion matrix of sLFP relative to a tetrode in a 5-class model (Fig.1C). Table 1 summarizes key differences between tetrodes and sLFP. All comparisons are statistically significant (Tukey's test).

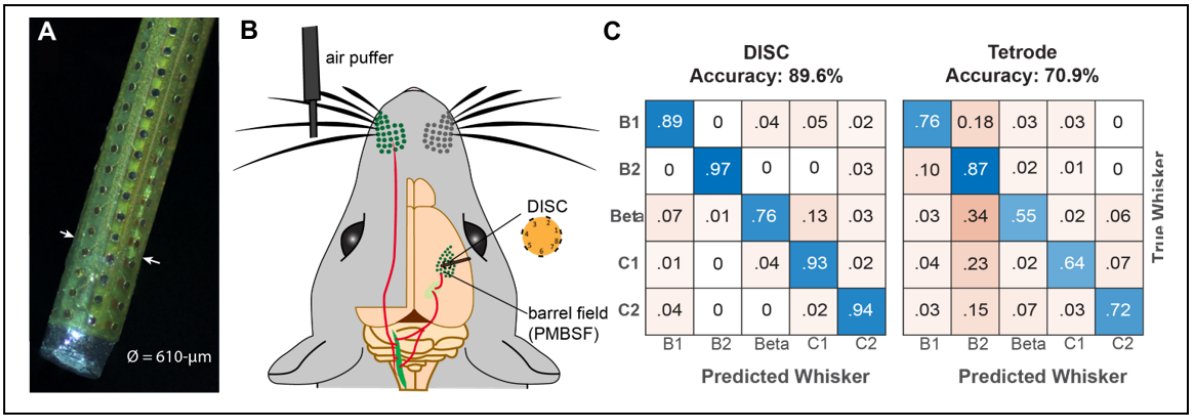


TABLE 1. 5-CLASS AND 9-CLASS WHISKER IDENTIFICATION (N=8)

Device	*Avg Waveform Amplitude, µV*	*5-Class Accuracy* [a]	*9-Class Accuracy*
MW/ Tetrode	95 ± 85 (SD)	68.7	52.0
sLFP	233 ± 214 (SD)	91.7	81.3

a. Whiskers classified: B1, B2, beta, C1, C2 and 9-class included D1, D2, gamma, delta

Disclosures: J. Seymour: A. Employment/Salary (full or part-time):; Rice University. **A. Abrego Mancilla:** None. **W. Khan:** None. **C. Wright:** None. **M. Ghajar:** None. **N. Tandon:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.02

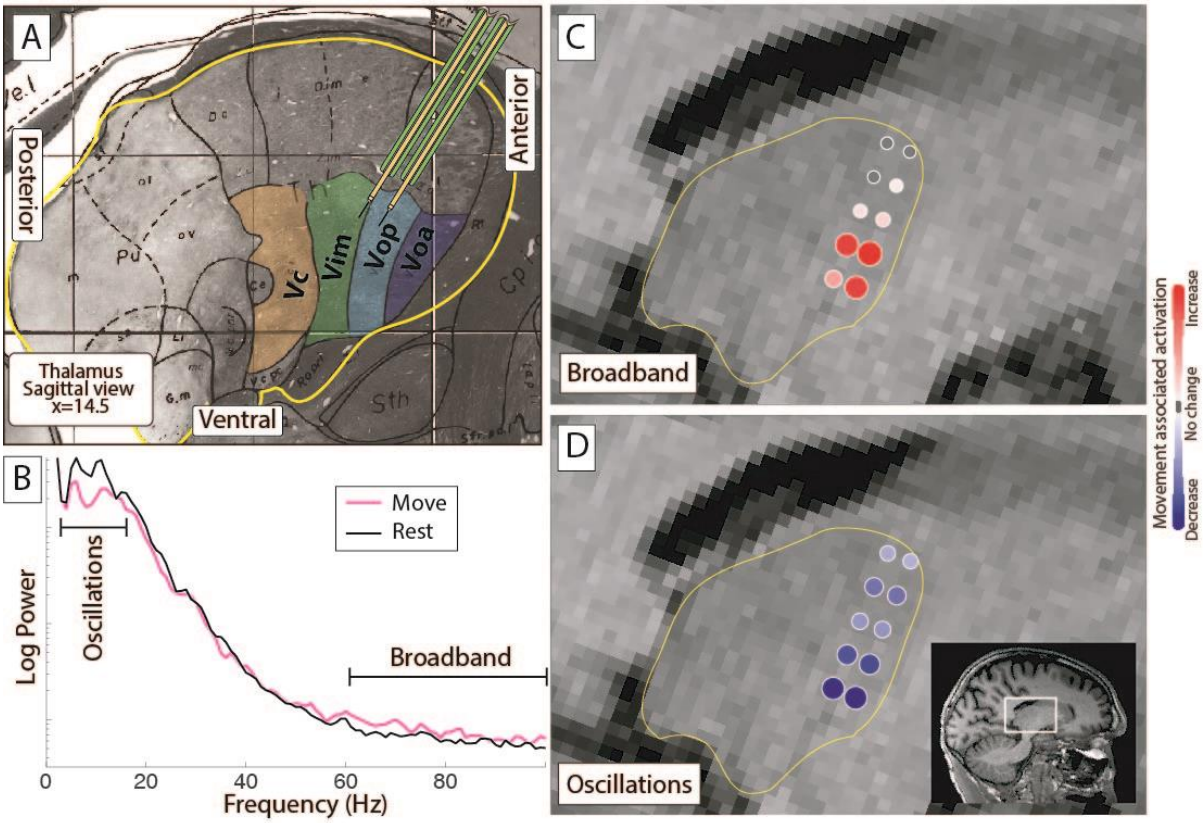
Topic: E.05. Brain-Machine Interface

Title: Task-dependent increase in broadband power is an electrophysiologic marker of focal thalamic activity

Authors: *B. T. KLASSEN, G. OJEDA VALENCIA, L. M. JACKSON, T. J. KAUFMANN, K. J. MILLER;
Mayo Clin., Rochester, MN

Abstract: The architecture of the human thalamus is currently indirectly inferred from human histopathologic and non-human animal studies. Given that movement-related increases in broadband power from electrocorticography recording have been previously shown to identify

specific motor cortical subregions involved with a particular movement, we explored whether this phenomenon can also be used to localize motor subregions of thalamus. We performed serial human thalamic local field potentials recordings using an electrode moved through multiple locations during deep brain stimulation surgery in 10 subjects. During the recording at each location, subjects alternated between 5 second epochs of rest and 5 second periods of contralateral hand movement. Power spectra were generated for each epoch using Welch's overlapping periodogram method (1 second window, 50% overlap). Spectra from all movement and rest epochs were separately averaged. The difference between the power in the 65 to 95 Hz band during movement versus rest was used as a proxy for task-related change in broadband activity. Task-related differences in prominent low-frequency oscillations (patient-specific sub-ranges within 5 to 30 Hz) were analyzed per individual oscillation and in aggregate. Significance was determined using unpaired T tests at a threshold of $p=0.05$. Sites of each recording were identified on each subject's MRI brain using the final DBS electrode implant site as a common point of reference. We find that recording sites showing statistically significant increases in broadband power during movement tend to tightly cluster in areas predicted to represent motor thalamus, whereas sites showing statistically significant reduction in the power (desynchronization) of low frequency oscillations are more diffusely distributed. This finding is replicated across all subjects. These results suggest that a task-dependent increase in broadband power is an electrophysiologic marker of focal thalamic activity and may be useful to delineate specific thalamic subregions on an individual subject basis.



Movement associated thalamic activation patterns: (A) Serial recordings were obtained from multiple thalamic locations during electrode decent towards the target in a motor subregion of thalamus (Vim) during deep brain stimulator placement in human subjects being treated for tremor. (B) Power spectra were computed at each recording site during subject hand movement (red) or rest (black). The difference between spectra during movement versus rest at each site were compared for both broadband activity (using 65-95 Hz band as a proxy) and bands containing patient-specific low-frequency oscillations (in this case 5 - 16 Hz). Each recording site was registered to the subject-specific brain MRI, and the distribution of sites with statistically significant differences (unpaired T test, p=0.05 threshold) was more spatially discrete for broadband shifts (C) than for low-frequncy oscillations (D).

Disclosures: B.T. Klassen: None. **G. Ojeda Valencia:** None. **L.M. Jackson:** None. **T.J. Kaufmann:** None. **K.J. Miller:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.03

Topic: E.05. Brain-Machine Interface

Support: NIH R01NS092882

Title: Living in the moment: Revisiting univariate and bivariate iEEG feature distribution dynamics across the circadian cycle

Authors: *I. BALZEKAS[1], V. KREMEN, JR[2], V. SLADKY[1], D. HERMES[1], G. A. WORRELL[1];
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Abstract: Background: Brain state dynamics are often studied with univariate and bivariate spectral features such as power in band (PIB) and coherence. The variability and nonstationarity of these data are managed by normalization and detrending, but these techniques may obscure meaningful dynamics, especially when comparing brain data with behavioral states occurring at broader timescales such as mood. With increasing interest in ambulatory brain recordings for behavioral decoding, we revisited the question of how electrographic features are distributed. **Objective:** The goal of this poster is to illustrate the distributions of common univariate and bivariate iEEG features across the circadian cycle throughout the brain. **Methods:** We applied standard pre-processing and feature calculation methods to intracranial electroencephalographic (iEEG) recordings from a patient who underwent iEEG monitoring for seizure onset zone localization. Relative PIB and coherence were calculated in 5, 10, and 30 second epochs from four days of continuous recording from 80 electrodes with concurrent visual sleep staging. No seizures occurred during this period. Electrodes from the seizure onset zone and epochs with epileptiform spikes were excluded from analysis. Each timeseries was used to build an overall feature distribution and a timeseries of feature distributions from 5-minute rolling windows. Mean, variance, skewness, and kurtosis were calculated for the overall and rolling feature distributions. **Results:** Overall relative PIB showed skewness ranging from -2 to 2 and kurtosis ranging from 0 to 5 depending on the electrode, band, and state. Although unbalanced with more samples available from the awake state, in the rolling window for relative PIB, skewness and kurtosis showed the largest shifts during sleep with NREM stages showing average increases in skewness and kurtosis compared to awake, with one contact in the frontal lobe, for example, showing significantly increased skew in the low and high gamma bands and kurtosis in the theta, beta, low gamma, and high gamma (Wilcoxon Rank-Sum, $p < 0.001$, $n1 = 684$, $n2 = 6778$). For many bipolar electrode pairs, coherence measurements wherein both electrode pairs had concurrent spectral peaks in the relevant band were relatively rare. **Conclusions:** Our illustration of the sensitivity of neural feature distributions to behavioral state highlights opportunities to consider the statistical properties of neural timeseries in conjunction with standard feature values. Characterizing iEEG statistical properties and dynamics at varying time scales should support robust experimental designs for behavioral sampling and decoding.

Disclosures: I. Balzekas: Other; Cadence Neuroscience Inc.. **V. Kremen:** None. **V. Sladky:** None. **D. Hermes:** None. **G.A. Worrell:** E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Cadence Neuroscience Inc., NeuroOne Inc..

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.04

Topic: E.05. Brain-Machine Interface

Support: Dutch Epilepsy Foundation 17-07

Title: Intra-operative SPES; the influence of propofol on local brain networks in epilepsy patients

Authors: *S. BLOK[1,2], D. VAN BLOOIJS[1], H. G. E. MEIJER[3], G. J. M. HUISKAMP[1], P. VAN EIJSDEN[1], F. S. S. LEIJTEN[1];
[1]Brain, UMC Utrecht, Utrecht, Netherlands; [2]Sci. and Technol., [3]Fac. of Electrical Engineering, Mathematics and Computer Science, Applied Analysis, Univ. of Twente, Enschede, Netherlands

Abstract: Background: Single Pulse Electrical Stimulation (SPES) can be performed in epilepsy patients with subdural electrocorticography to reveal underlying brain networks. Neural mass models (NMMs) can simulate such an individual brain network. In the future, we want to explore the use of a NMM-based replica of the patient´s brain to evaluate various resection strategies during surgery. This approach could significantly reduce long-term intracranial monitoring or even make it redundant. We then have to know the influence of anesthesia (propofol) on these networks. Method: In a retrospective analysis (n=6), we compared SPES networks derived from two and ten stimuli. We calculated the total, positive and negative agreement. We compared the absolute number of Early Responses (ERs), the in-degree, out-degree and betweenness centrality. In an experimental study (n=6), we performed SPES twice: during long-term intracranial EEG monitoring, using ten stimuli and under propofol anesthesia at grid-explantation, using two stimuli. We calculated the above network parameters as well as the N1- and N2-peak latencies of ERs. Finally, we titrated coefficients (gain and time constants) of the NMM to simulate the change of N1 and N2 responses during propofol. Results: The network parameters calculated for the retrospective analysis showed a significant correlation ($p<0.05$) between the two stimuli and ten stimuli setting for five out of six patients, while for the experimental study we found a significant correlation in four out of six patients. All network parameters showed, on average, a lower value during propofol-SPES. We observed increased peak-latencies during propofol. This was simulated in a NMM by decreasing the gain and increasing the time constant of fast inhibition. Conclusion: Networks derived from two instead of ten stimuli per stimulation pair are similar. Effective connectivity based on SPES remains similar during propofol anesthesia.

Modulating the fast inhibition in the NMM can simulate the experimentally observed effect of propofol on ERs.

Disclosures: S. Blok: None. **D. Van Blooijs:** None. **H.G.E. Meijer:** None. **G.J.M. Huiskamp:** None. **P. van Eijsden:** None. **F.S.S. Leijten:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.05

Topic: E.05. Brain-Machine Interface

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U01-NS108916)
NIH/NINDS (U24-NS109103)
NIH/NIHMH (R01-MH122258)
NIH/NCATS (KL2TR002379)
Fondazione Neurone

Title: BIC-BCI2000: A general-purpose hardware and software platform for chronic intracranial neuromodulation

Authors: *A. BELSTEN[1], *A. BELSTEN[1,2], G. SCHALK[3], S. WORRELL[3], F. MIVALT[3], I. KIM[3], S. MESSINA[3], T. KAUFMANN[3], N. STAFF[3], D. HERMES[3], G. A. WORRELL[3], K. MILLER[3], P. BRUNNER[1,2];
[1]Washington Univ. in St. Louis Sch. of Med., St. Louis, MO; [2]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [3]Mayo Clin., Rochester, MN

Abstract: Neuromodulation using intracranial electrical stimulation is effective in the therapeutic treatment of epilepsy, Parkinson's, essential tremor, and depression. However, contemporary neuromodulation devices depend on having a fundamental understanding of the beneficial effect of electrical stimulation on the underlying electrophysiological pathology, and thus provide limited benefit in developing therapies for less well-understood neurological disorders and diseases. Our study aimed to overcome this limitation by developing a general-purpose hardware and software platform for neuromodulation research and development based on the CorTec Brain Interchange (BIC) and the BCI2000 general-purpose software platform. The BIC is a fully implantable and inductively powered neuromodulation device intended for human use. It supports recording and stimulation through 32 electrodes. While the BIC supports autonomous operation (i.e., rapid stimulation based on prevalent signal conditions), it also allows

for streaming of the recorded signals to external applications to analyze signals and initiate stimulation in real time. These two operation modes facilitate the entire range of scientific and clinical investigation of neuromodulation therapies, ranging from the formulation of the scientific hypothesis, over initial validation of the therapeutic intervention, to the eventual clinical trial. Our study worked towards this goal by developing an integrated BIC-BCI2000 system. We implanted the BIC system in a beagle canine. Two AirRay 16-channel grids were subdurally placed over the left and right sensorimotor cortex via a bilateral craniotomy. Over the 77 days of implantation, we performed electrocorticographic (ECoG) recordings in the behaving and asleep canine using the BCI2000 software. Analysis of the recorded ECoG signals revealed the expected oscillatory activity in the beta band. This initial demonstration of the BIC-BCI2000 system demonstrates the viability of general-purpose hardware and software platforms for neuromodulation research and development.

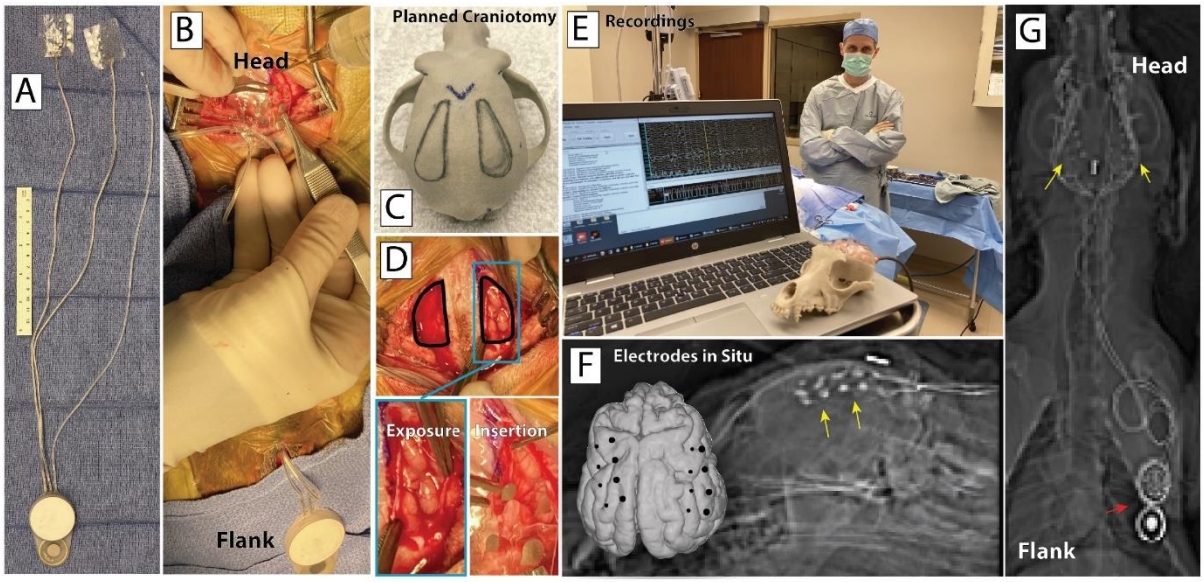


Figure: A) BIC and two 16-channel electrode grids. **B)** Electrodes placed subdurally on the cortex; BIC device placed behind the scapula. **C&D)** (C) Planned and (D) performed bilateral craniotomy, exposed cortex, and electrode grid insertion. **E)** Neural data streaming to BCI2000. **F)** Co-registered pre-operative MRI and post-operative CT highlight electrode locations. **G)** Post-operative antero-posterior x-radiograph showing electrode locations and BIC device.

Disclosures: A. Belsten: None. **G. Schalk:** None. **S. Worrell:** None. **F. Mivalt:** None. **I. Kim:** None. **S. Messina:** None. **T. Kaufmann:** None. **N. Staff:** None. **D. Hermes:** None. **G.A. Worrell:** None. **K. Miller:** None. **P. Brunner:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.06

Topic: E.05. Brain-Machine Interface

Support: UG3-NS112826 Thalamic stimulation to prevent impaired consciousness in epilepsy (START)

Title: A Wearable and Mobile Health Platform for Automatic Responsiveness Testing in Epilepsy (ARTiE): An EMU Pilot Study

Authors: *L. WHEELER[1,2,3], V. KREMEN, JR[2,3], K. MCQUOWN[5], V. SLADKY[2], B. BRINKMANN[2,3], Z. AHMAD[6], C. YOTTER[6], C. BENJAMIN[6], J. GIACINO[7], B. C. JOBST[8], G. A. WORRELL[4], H. BLUMENFELD[9];
[1]Mayo Clin. Grad. Sch. of Biomed. Sci., Rochester, MN; [2]Neurol., [3]Physiol. and Biomed. Engin., [4]Mayo Clin., Rochester, MN; [5]Windy City Labs, Chicago, IL; [6]Neurol., Yale Univ., New Haven, CT; [7]Physical Med. and Rehabil., Spaulding Rehabil. Hosp., Boston, MA; [8]Neurol., Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [9]Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Rationale: Alteration of consciousness (AOC) during seizures is a hallmark of epilepsy, leading to increased morbidity, mortality, and reduced quality of life. Assessment of AOC level is crucial to accurate diagnosis and management of people with epilepsy (PWE). However, current AOC testing during seizures has mainly been limited to hospital settings, and is further limited by a lack of standardization, sparse testing, delay to testing, poor interrater and test-retest reliability. The absence of a standardized AOC assessment tool for testing PWE in naturalistic environments has limited investigation of AOC mechanisms and its impact on patients. To address this clinical gap, we develop an application for automated responsiveness testing in epilepsy (ARTiE) utilizing a smartphone and smartwatch. **Methods:** The ARTiE system was validated in the Epilepsy vEEG Monitoring Unit. Patient inclusion criteria were: 1) epilepsy diagnosis; 2) age 18 years or older; 3) ability to participate in behavioral tasks at baseline. The ARTiE system (Apple iPhone, Apple watch & cloud) administers the behavioral assessment sequence and records verbal and motor responses. The devices have bidirectional wireless connectivity allowing remote assessment initiation and data synchronization. Upon automated or visual seizure detection a remote server notification automatically launches testing that includes verbal questions and commands to test receptive and expressive language, orientation, motor praxis, and memory recall. The assessment adaptively increases the number of loops based on patient performance. **Results:** Thirteen adult PWE were enrolled. A total of 56 baseline, 6 clinical, and 9 subclinical automated triggered events were captured testing motor, memory, and orientation in PWE. During baseline testing, subjects achieved 98.5% for motor task, 100% for memory registration, 100% for orientation, and 87.5% memory retrieval performance. There were no significant differences between baseline and subclinical seizure testing. Clinical seizures showed reductions of 10.7% in motor, 10.5% in verbal, and 20.8% memory retrieval tasks compared to baseline. Generalized tonic-clonic seizure testing showed maximum impairment at testing initiation with 0% memory registration, 0% motor task, and 0% orientation task completion. The subject returned to 100% motor task, 100% orientation, and 0% memory retrieval by the end of testing. The subjects reported they enjoyed working with the

technology and are interested in future studies in their home environment. Future applications include integration of ARTiE with a fully implantable neurostimulation and sensing device.

Disclosures: L. Wheeler: None. **V. Kremen:** None. **K. McQuown:** None. **V. Sladky:** None. **B. Brinkmann:** None. **Z. Ahmad:** None. **C. Yotter:** None. **C. Benjamin:** None. **J. Giacino:** None. **B.C. Jobst:** None. **G.A. Worrell:** None. **H. Blumenfeld:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.07

Topic: E.05. Brain-Machine Interface

Support: NIH/NINDS Brain Initiative NIH UH3 NS095495 (Neurophysiologically Based Brain State Tracking & Modulation in Focal Epilepsy)
The authors are grateful for advice and discussion made possible via the OpenMind Consortium, an effort supported by BRAIN Initiative resource dissemination grant U24NS113637.

Title: Circadian Rhythms of Human Brain - Electrical Impedance

Authors: *F. MIVALT[1,5], V. SLADKY[1,6,7], I. BALZEKAS[1], P. NEJEDLY[1], T. PRIDALOVA[1,5,7], B. H. BRINKMANN[1], N. GREGG[1], B. N. LUNDSTROM[1], S. MESSINA[2], T. DENISON[8], D. HERMES MILLER[3], K. MILLER[4], J. VAN GOMPEL[4], V. KREMEN[1,6,9], G. A. WORRELL[1,3];
[1]Neurol., [2]Neuroradiology, [3]Physiol. and Biomed. Engin., [4]Neurosurg., Mayo Clin., Rochester, MN; [5]Dept. of Biomed. Engineering, Fac. of electrical Engin. and Communication, Brno Univ. of Technol., Brno, Czech Republic; [6]Fac. of Biomed. Engin., Czech Tech. Univ. in Prague, Kladno, Czech Republic; [7]Intl. Clin. Res. Center, St. Anne's Univ. Hosp., Brno, Czech Republic; [8]The Inst. of Biomed. Engineering, Univ. of Oxford, Oxford, United Kingdom; [9]11.Czech Inst. of Informatics, Robotics, and Cybernetics, Czech Tech. Univ. in Prague, Prague, Czech Republic

Abstract: Rationale: Long-term data collection using implantable neural sense and stimulation (INSS) devices in patients with epilepsy enables investigation of ultradian, circadian and infradian rhythms in sleep, seizures, interictal epileptiform discharges (IED), and brain impedance. It has long been recognized that IED and seizures have circadian patterns. Recent studies also highlight the importance of circadian changes in brain extracellular space, and the functional role of slow-wave sleep and the associated increase in extracellular space volume that is hypothesized to provide improved metabolite and protein clearance. The direct evidence for increased extracellular space volume during sleep primarily comes from rodent studies, and a

few human imaging studies with limited temporal sampling. Here we investigate a potential surrogate of extracellular volume, the brain electrical impedance in hippocampus (HPC) and anterior nucleus of thalamus (ANT) over multiple months in four ambulatory humans with drug resistant epilepsy.
Methods: We collected several months of continuous iEEG and impedance measurements from each of 4 patients with drug resistant epilepsy implanted with an investigational INSS and living in their normal home environments. Continuous iEEG was used to precisely assess sleep states using machine learning algorithms. Impedance was measured in 15-minute intervals in bilateral ANT and HPC to analyze the relationship between sleep states and impedance cycles (Pearson's Correlation Coefficient and Mann-Whitney U test) using 31 days and nights of data.
Results: The ANT and HPC electrical impedance followed a distinct circadian rhythm with period ranging between 23.36 and 24.81 hours. We observed a phase shift in the maximum and minimum between ANT and HPC impedance with Pearson's Correlation Coefficient of 0.604 (p &lt .001), while the left and right HPC shows a high correlation 0.700 (p &lt .001) without any phase shift. Moreover, impedances of both HPC and ANT shows a distinct correlation with sleep (0.504 and 0.507 both p &lt .001) with the average absolute impedance change, across all measured leads, between wake and sleep $26.3 \pm 13.2\ \Omega$ (p &lt .001).
Conclusion: We show human brain impedance cycles with a circadian rhythm that varies across different behavioral states and brain locations. Further research of this phenomena and the relationship of impedance to sleep, seizures, epileptiform interictal discharges may prove useful for understanding the role of the extracellular space in seizure generation in temporal lobe epilepsy.

Disclosures: F. Mivalt: None. **V. Sladky:** None. **I. Balzekas:** A. Employment/Salary (full or part-time):; Cadence Neuroscience Inc.. **P. Nejedly:** None. **T. Pridalova:** None. **B.H. Brinkmann:** 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.; Cadence Neuroscience Inc., UNEEG Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNEEG, NeuroOne, Epiminder, Medtronic, Philips Neuro. F. Consulting Fees (e.g., advisory boards); UNEEG, NeuroOne, Epiminder, Medtronic, Philips Neuro. **N. Gregg:** 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.; UNEEG Inc. **B.N. Lundstrom:** 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.; Cadence Inc., Medtronic, Neuropace. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNEEG, NeuroOne, Epiminder, Medtronic, Philips Neuro. F. Consulting Fees (e.g., advisory boards); UNEEG, NeuroOne, Epiminder, Medtronic, Philips Neuro. **S. Messina:** None. **T. Denison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent licensed to Medtronic. **D. Hermes Miller:** None. **K. Miller:** None. **J. Van Gompel:** 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.; Cadence Neuroscience Inc.. **V. Kremen:** None. **G.A. Worrell:** 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.; Cadence Neuroscience Inc., UNEEG Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNEEG Inc., NeuroOne, Epiminder, Medtronic, Philips Neuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne Inc.. F. Consulting Fees (e.g., advisory boards); UNEEG Inc., NeuroOne, Epiminder, Medtronic, Philips Neuro.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.08

Topic: E.05. Brain-Machine Interface

Support: NIH - NIMH Grant R01MH122258

Title: Electrical stimulation responses in human ventral temporal cortex cluster by shape and stimulation site on intracranial EEG

Authors: *H. HUANG[1], N. M. GREGG[2], G. OJEDA VALENCIA[3], B. H. BRINKMANN[2], B. N. LUNDSTROM[2], G. A. WORRELL[2], K. J. MILLER[4], D. HERMES[3];
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Abstract: Motivation
Human ventral temporal cortex (VTC) is highly connected to serve its function in object perception. Electrical brain stimulation can be used to study how inputs from other brain regions drive responses in the VTC. To map the variability of inputs, we recorded intracranial EEG (iEEG) in human VTC in response to single pulse stimulation of other regions and we clustered responses by shape and anatomical location of stimulation site.
Methods
We recorded iEEG data in 7 patients who had been implanted for epilepsy monitoring. We stimulated pairs of electrodes at multiple sites in the brain with a single pulse every 5 to 7s and measured corticocortical evoked potential (CCEP) responses within the collateral sulcus and lateral occipitotemporal sulcus of the VTC. Using a novel unsupervised machine learning method, we uncovered 2 to 4 distinct response shapes, which we term basis profile curves (BPCs), from the 20 to 500 ms post-stimulation interval and characterized the anatomical

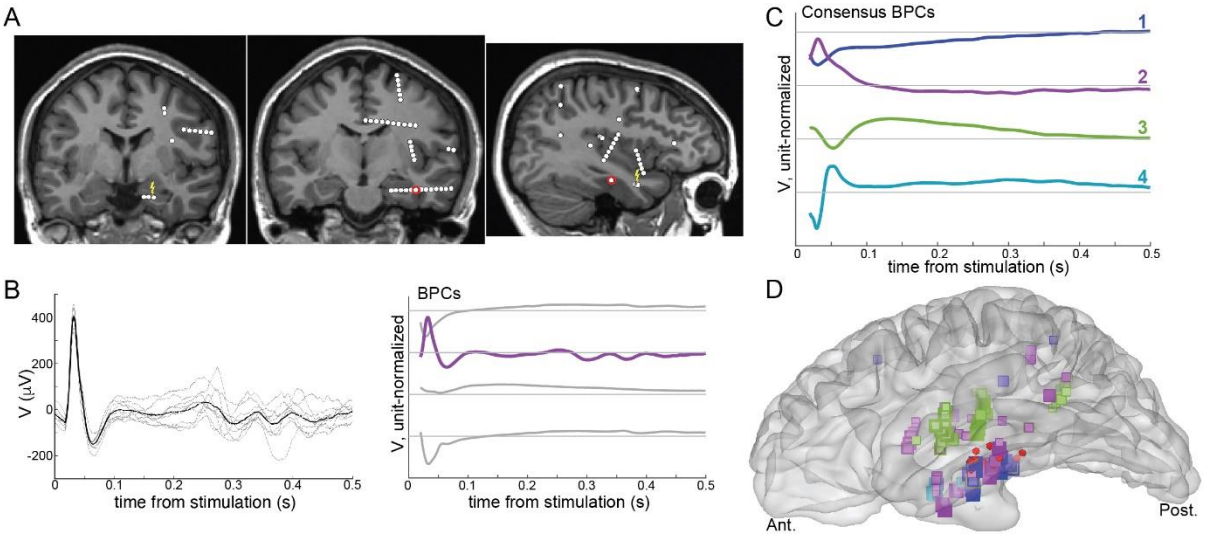
distribution of stimulation sites that cluster to each BPC.

Results

IEEG recordings from both regions of the VTC showed evoked potentials of high amplitude following stimulation of several other regions. Some of the BPCs concentrated at amygdala, anterior hippocampus, and insula, while another BPC concentrated at the posterior hippocampus. Stimulation of lateral cortical sites, such as at middle temporal gyrus, evoked yet another BPC. We tested the model's between-subject reliability by calculating consensus BPCs using collateral sulcus responses from 5 subjects and then predicting the responses from a 6th subject. We found that the anatomical distribution of consensus BPCs in the 6th subject largely matched that of the first 5. There was greater between-subject variability when recording in the lateral occipitotemporal sulcus.

Conclusion

Characterizing distinct shapes in stimulation responses provides a novel description of connectivity in the VTC. We derive connectivity maps that reveal different inputs from cortical and limbic structures.



From stimulation responses in one subject to consensus BPCs across 5 subjects. (A) T1-weighted MRI slices from subject 1 illustrating one recording site in the collateral sulcus (red) and one stimulation site in the amygdala (yellow). (B) Left: individual trials (gray) and mean across trials (black) of stimulation-recording pair in (A). Right: BPCs at recording site in (A) calculated from all stimulation site responses. Responses to amygdala stimulation cluster to the BPC highlighted in purple. (C) Consensus BPCs from 5 subjects calculated by k-means clustering of all subject BPCs. (D) Anatomical distribution of stimulation sites from 5 subjects projected onto an MNI brain, colored and scaled by consensus BPC cluster and strength.

Disclosures: H. Huang: None. **N.M. Gregg:** None. **G. Ojeda Valencia:** None. **B.H. Brinkmann:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence. **B.N. Lundstrom:** 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.; Medtronic EPAS, NeuroPace RESPONSE Study, Neuroelectrics tDCS for Patients with Epilepy Study. **G.A. Worrell:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); Cadence. **K.J. Miller:** None. **D. Hermes:** None.

Poster

P548. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P548.09

Topic: E.05. Brain-Machine Interface

Support: NIH NIMH R01MH122258

Title: A validated BIDS app for the automated detection of early evoked responses and mapping of connectivity in IEEG

Authors: *M. A. VAN DEN BOOM[1], D. VAN BLOOIJS[4], N. M. GREGG[2], G. OJEDA VALENCIA[1], B. N. LUNDSTROM[2], G. A. WORRELL[2], J. F. VAN DER AAR[5], G. J. M. HUISKAMP[4], K. J. MILLER[3], F. S. S. LEIJTEN[4], D. HERMES[1];
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Abstract: Several studies have combined intracranial monitoring with single pulse electrical stimulation to map and characterize connectivity in the human brain. Through systematic stimulation of electrode pairs with a brief pulse, early responses can be identified on other electrodes, indicating connectivity between the stimulated and measured site. While standardized approaches are essential for scientific reproducibility and automated inclusion in clinical workflows, the methods that are used to identify response profiles vary between studies, with each applying their own criteria. Some studies rely purely on the visual inspection of the average response signal, whereas others apply some form of automatic detection. The extension of the BIDS standard to include intracranial EEG (iEEG) now allows us to standardize this process and we present an open-source BIDS app that performs automatic detection of early evoked responses (i.e. the deflection within the first 100ms after stimulation, also known as N1 or P1). The output of the app was validated against visually inspected and manually annotated data of 14 participants recorded at two different hospitals. The initial detection algorithm that relies on the identification of relative local extremums in the average signal, provided a detection accuracy of around 90%. Depending on the context, more strict detection (e.g. in a clinical setting) or less strict detection (e.g. in a research setting) could be applied. As such, the app provides the ability to adjust various detection parameters and produces two additional output metrics that can be used to further raise the sensitivity or specificity, or serve as an alternative detection input value. One metric provides a measure of response consistency (i.e. inter-trial reliability), whereas the

other metric gives an indication on the typicality of the response shape. The app produces data-files and visualizations of the detection results per channel and per stimulated electrode-pair. In addition, connectivity visualizations are generated that allow for easy visual inspection of connectivity. Altogether, this BIDS app provides automated methods that could serve as a standardized and easy-to-use tool for a reliable detection of early evoked responses in both clinical and research settings.

Disclosures: M.A. van den Boom: None. **D. Van Blooijs:** None. **N.M. Gregg:** None. **G. Ojeda Valencia:** None. **B.N. Lundstrom:** None. **G.A. Worrell:** None. **J.F. van der Aar:** None. **G.J.M. Huiskamp:** None. **K.J. Miller:** None. **F.S.S. Leijten:** None. **D. Hermes:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.01

Topic: E.05. Brain-Machine Interface

Support: NEI F30EY032799
NINDS U01NS099724
NIGMS T32 GM008042
Tianqiao and Chrissy Chen Institute

Title: Functional ultrasound neuroimaging reveals heterogenous organization of directional response fields in lateral intraparietal cortex of nonhuman primates

Authors: *W. S. GRIGGS[1], S. L. NORMAN[1], C. RABUT[2], C. DEMENÉ[5], V. N. CHRISTOPOULOS[3,6], M. TANTER[5], M. SHAPIRO[2], R. A. ANDERSEN[4];
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Abstract: The posterior parietal cortex (PPC) is an important area for the transformation of spatial information into accurate motor movements. PPC sub-regions such as the lateral intraparietal area (LIP) and the parietal reach region (PRR) are especially important for saccades and reaches, respectively. However, our understanding of these areas' functional organization is incomplete, in part due to technical challenges. While electrophysiology recordings can only record from small regions of the PPC at a time, fMRI and other whole-brain techniques have insufficient spatiotemporal resolution. Now, functional ultrasound imaging (fUSI) is available as an innovative neuroimaging technique that measures cerebral hemodynamics with exceptional spatiotemporal resolution (100 μm and up to 10 ms) and a large field of view (several cm) (Macé et al., *Nature Methods*, 2011). These specifications are ideally suited to recording detailed

activity of multiple cortical regions in parallel in nonhuman primates (Norman et al., *Neuron*, 2021). In this study, we used fUSI to record changes in cerebral blood volume (CBV) in PPC of two awake behaving nonhuman primates. Each animal performed memory-guided saccades to targets throughout their visual field. We analyzed the distribution of response fields within each coronal plane of PPC. We found that many subregions within and across coronal LIP planes had different directional tuning, although predominately for the contralateral space. We also found substantially more activity in LIP compared to PRR during our saccade task, supporting the respective roles of LIP and PRR for eye and hand movements. These results address a fundamental gap in our understanding of LIP's functional organization by developing mesoscopic maps of direction specificity previously unattainable with fMRI or electrophysiology methods. These mesoscopic maps reveal a highly heterogeneous organization within LIP with many small patches of neighboring cortex encoding different preferred directions.

Disclosures: W.S. Griggs: None. **S.L. Norman:** None. **C. Rabut:** None. **C. Demené:** None. **V.N. Christopoulos:** None. **M. Tanter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus. **M. Shapiro:** None. **R.A. Andersen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.02

Topic: E.05. Brain-Machine Interface

Support: NIH R01EY015545
NIH/NIE UG1EY032039
NIH/NRSA T32NS105595
The Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
The Boswell Foundation

Title: Distinct patterns of whole body coding in motor cortex and posterior parietal cortex during neural prosthetic control

Authors: *K. KADLEC[1], T. AFLALO[1], J. A. GAMEZ[1], E. ROSARIO[2], N. POURATIAN[3], R. A. ANDERSEN[1];
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Abstract: The segregation of motor plans based on the part of the body to be moved (effector) is classically thought to occur early in the plan's formation. For example, in posterior parietal cortex (PPC) of non-human primates, studies show distinct functions across sub-regions, such as

coding for eye movements in lateral intraparietal area and hand grasp movements in the anterior intraparietal area. The separation of effectors has also been observed in motor cortex (MC), where stimulation and neuroimaging studies suggest the organization of the body from head to toe is from lateral to medial cortex respectively. Recent findings from human single-neuron studies have revealed much more diversity in action representation than expected in both PPC and MC, with multiple effectors being encoded in small populations. These results are difficult to interpret together in the context of previous findings. Further, comparing different brain regions from different studies is difficult given variability both between individuals and behaviors. In the current study, we simultaneously recorded from single neurons in human PPC and MC (hand knob) of a tetraplegic patient enrolled in an ongoing brain-machine interface study. The participant attempted 2-D movements using 12 effectors (head to foot, left and right side). PPC demonstrated nearly equivalent, though still discriminable, coding for all tested body parts. In contrast, MC showed a clear preference for the contralateral distal hand, as expected from anatomy, with reduced, albeit significant, tuning to the other effectors, bilaterally. In a subsequent experiment, we found that movement representations for multiple body segments were preserved even when the causal impact of this activity could drive task errors (i.e. unwanted movements). This suggests that coding for multiple effectors is a robust feature of the recorded neural populations. Such robust encoding suggests that our findings in a tetraplegic individual may be representative of motor-intact individuals, but further work is needed to confirm this. Overall, the results show greater overlap in effector coding than anticipated in both regions, especially in PPC where the strength of representation was comparable across the entire body.

Disclosures: K. Kadlec: None. **T. Aflalo:** None. **J.A. Gamez:** None. **E. Rosario:** None. **N. Pouratian:** None. **R.A. Andersen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.03

Topic: E.05. Brain-Machine Interface

Support: NIH/NEI Grant UG1EY032039
NIH/NEI Grant R01EY015545
Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
Amazon AI4Science Fellowship
Boswell Foundation

Title: Preserved motor representations enable neural prosthetic control of individual fingers

Authors: *C. GUAN[1], T. AFLALO[1], C. Y. ZHANG[1], E. ROSARIO[2], N. POURATIAN[3], R. A. ANDERSEN[1];
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Abstract: Paralyzed individuals can control prosthetics using brain-machine interfaces (BMIs), even long after spinal cord injury. What neural mechanisms enable this BMI control? Do BMI users activate preserved motor circuits, or have they learned novel activity patterns specific to BMI control? We first demonstrate that a tetraplegic human participant can control an individual-finger BMI through intracortical recordings from the posterior parietal cortex. Control performance exceeded previously known results. We then show that the neural representational structure of these attempted finger movements matches the representational structure previously measured in able-bodied subjects. Furthermore, we identify multiple functional processes that dissociate in time, first encoding motor-production-like representations, which are followed approximately 170ms later by sensory-like representations. This pattern is consistent with PPC encoding a state estimate of the body. Our results suggest that pre-existing neural representations are preserved after injury. BMI users can then reengage these preserved representations during neural prosthetic control. Future neural prosthetics may be able to extend functionality by leveraging the structure of these able-bodied-like representations.

Disclosures: C. Guan: None. **T. Aflalo:** None. **C.Y. Zhang:** None. **E. Rosario:** None. **N. Pouratian:** None. **R.A. Andersen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.04

Topic: E.05. Brain-Machine Interface

Support: NEI R01EY015545
NEI UG1EY032039
Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
Boswell Foundation

Title: Neural prosthetic context profoundly shapes single-neuron responses while preserving the functional distinctions between human motor and posterior parietal cortices.

Authors: *T. AFLALO[1], J. A. GAMEZ[1], K. KADLEC[1], S. SAKELLARIDI[1], C. GUAN[1], E. ROSARIO[2], N. POURATIAN[3], R. A. ANDERSEN[4];
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Centers For Healthcare, Pomona, CA; [3]UTSouthwestern, Dallas, TX; [4]Div. of Biol. and Biol. Engin., Calif Inst. of Technol., Pasadena, CA

Abstract: A classic perspective is that distinct functions of the brain are organized into separate regions of cortex. However, recent work complicates this view; e.g., neurons in primary visual cortex correlate with movements in the dark, and small patches of cortex respond to all parts of the body. These discrepancies in the functional organization of cortex are relevant for neural prosthetic applications. Is it necessary to target specific cortical areas to harness specific functional signals, or does the intrinsic diversity of cortical signals combined with plasticity and novel demands blur distinctions between cortical regions?
Here, we study neural population coding of motor plans in the human posterior parietal cortex (PPC) and motor cortex (MC) during neural prosthetic control of a computer cursor. These regions are associated with motor planning and execution, respectively, providing a testbed to probe functional specialization. We tested planning activity across three experimental contexts: planning 1) while the cursor was locked in place (cursor *decoupled* from brain activity), 2) while the cursor was free to move, but the task enforced a hold period (cursor *coupled* to brain activity), and, 3) during continuous *sequential* movements in which the participant planned the next movement simultaneous with the current movement execution.
We found that context profoundly shapes neural responses in both PPC and MC. When neural activity during planning is *decoupled* from cursor movement, MC shows comparable levels of planning activity to PPC. When neural activity is *coupled* to cursor movement, MC activity drops 3-fold relative to PPC. Finally, during *sequential* movements, there was no measurable planning activity in MC, while planning in PPC persisted. Thus, while MC can express planning activity, this activity is fragile, and disappears under contextual pressures. PPC is also shaped by context. In *coupled* and *decoupled* contexts, population encoding during planning and execution were functionally overlapping (e.g. population codes were highly correlated). However, in the *sequential* task, PPC planning and execution neural subspaces orthogonalized, minimizing cross-talk between movement representations. Similar to PPC, MC planning and execution activity were highly overlapping in the *decoupled* and *coupled* contexts.
Our results demonstrate clear functional distinctions between cortical regions despite the novel functional demands imposed by the neural prosthetic system. However, task context strongly shapes neural populations and may account for reported discrepancies in the functional organization of cortex.

Disclosures: T. Aflalo: None. **J.A. Gamez:** None. **K. Kadlec:** None. **S. Sakellaridi:** None. **C. Guan:** None. **E. Rosario:** None. **N. Pouratian:** None. **R.A. Andersen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.05

Topic: E.05. Brain-Machine Interface

Support: C14\18\100

Title: Decoding reaching direction from macaque dorsal and ventral premotor and primary motor cortex

Authors: J. G. RAMIREZ[1], S. DE SCHRIJVER[3], T. DECRAMER[2], T. THEYS[4], ***P. JANSSEN**[1];
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Abstract: Primary and premotor cortex are involved in visually guided motor behavior. To compare the decoding performance of reaching movements in premotor and primary motor cortex, we implanted three 96-channel Utah arrays in ventral premotor area F5c, dorsal premotor area F2 and primary motor cortex M1 in two macaque monkeys that were trained to perform a 3D center-out task on a touchscreen. Using DeepLabCut, we tracked the position of the wrist while the monkeys performed the task, and correlated this with the neural activity. We propose a nonlinear extension of a recent algorithm for modelling neural activity (Preferential Subspace Identification or PSID) to develop a high-performance brain-machine interface. In our method, we first extracted latent neural dynamics using PSID. Then, we applied a nonlinear transformation to the extracted dynamics, after which the transformed dynamics were used to build a regression model to predict the behavioral states at each 50 ms timestep. When tested with two rhesus monkeys performing a 3D center-out task, our approach has yielded highly accurate decoding ($r^2 = 0.88$) while having a reduced latency (order of microseconds) making it convenient for online decoding. Moreover, we have obtained promising decoding results using the neural activity in a passive observation task with a simulated robot in a 3D virtual environment performing the same 3D center out task. Finally, we tested the decoding capacity of each individual area using our method in one of the monkeys. We obtained comparable peak performance in M1 ($r^2 = 0.87$) and PMd ($r^2 = 0.85$) and lower though significant decoding accuracyin the F5c sector of PMv ($r^2 = 0.52$). Thus, robust and fast decoding of reaching movements is possible from neural activity recorded in PMd, M1 and to a lesser extent PMv.

Disclosures: J.G. Ramirez: None. **S. De Schrijver:** None. **T. Decramer:** None. **T. Theys:** None. **P. Janssen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.06

Topic: E.05. Brain-Machine Interface

Support: EFRE IT- 2-2-023 (VAFES)

Title: Beyond error correction to improve brain-computer interfaces using error-related potentials

Authors: *A. XAVIER FIDÊNCIO[1,2], T. GLASMACHERS[4], C. KLAES[3], I. IOSSIFIDIS[1]; [1]Inst. of Computer Sci., Ruhr West Univ. of Applied Sci., Mülheim an der Ruhr, Germany; [3]Dept. of Neurosurg., [2]Knappschaftskrankenhaus Bochum, Bochum, Germany; [4]Inst. for Neural Computation, Ruhr-Universität Bochum, Bochum, Germany

Abstract: Error-related potentials (ErrPs) are known to be elicited in the human brain during the brain-computer interaction as a direct reaction to not only self-made errors, but also to mistaken actions performed by an external system. Current literature on non-invasive BCIs refers to seven different ErrPs and show that these signals can be generated under different experimental paradigms. This has motivated an increased interest over the last years in the integration of these signals into different BCI systems to improve their performance, since the ErrPs provide a convenient source of feedback to the system with no extra workload being given to the subject. Several works have successfully shown the improvement achieved while using the ErrPs signals to undo the perceived error in different systems. However, more research is still necessary regarding the use of the decoded error information going beyond just error correction. For this reason, we investigate alternative strategies to integrate the ErrPs in different BCI systems to improve their overall performance. These error signals can be reliably measured using Electroencephalography (EEG) and usually wet electrodes are applied. In this work, however, we record EEG data using a Brain Products LiveAmp amplifier and the Xpress Twist cap with 32 active dry electrodes, which decreases experiment preparation time, but imposes new challenges regarding the signal-to-noise ratio.

Our first approach is focused on gesture-based BCIs using Electromyography (EMG) data as input, in which different supervised learning strategies triggered by the ErrPs to adapt an existing EMG-decoder to a new subject are tested. Additionally, we investigate the integration of the ErrPs as reward signal in a reinforcement learning framework to learn the correct mapping between measured input signal to BCI control commands. Simulation results show the feasibility of this approach for different ErrPs decoding accuracies in an EMG-based BCI. Preliminary results on online application including two subjects is, however, so far limited by the low ErrPs decoding accuracy of around 60%.

Hence, while error signals are an important source of additional and relevant information for BCIs, their effective use is highly dependent on their accurate detection and classification. Therefore, we additionally investigate the application of different machine learning algorithms such as regularized linear discriminant analysis (rLDA), support vector machine (SVM) and more complex neural network-based models using time- and/or frequency domain features to reliably decode the ErrPs signals in a single-trial basis.

Disclosures: A. Xavier Fidêncio: None. **T. Glasmachers:** None. **C. Klaes:** None. **I. Iossifidis:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.07

Topic: E.05. Brain-Machine Interface

Support: The T & C Chen Brain-machine Interface Center at Caltech
The Boswell Foundation
NIH BRAIN U01NS099724
The Della Martin Foundation

Title: Fusinet - an artificial neural network for generalized decoding of movement intention using functional ultrasound neuroimaging

Authors: *S. L. NORMAN[1], W. S. GRIGGS[1], C. RABUT[1], D. MARESCA[2], V. N. CHRISTOPOULOS[3], C. DEMENÉ[4], M. TANTER[4], M. G. SHAPIRO[1], R. A. ANDERSEN[5]; [1]Caltech, Pasadena, CA; [2]Delft Univ. of Technol., Delft, Netherlands; [3]Div. of Biol. and biological Engin., Univ. of California, Riverside, Riverside, CA; [4]Physics for Med. Paris, INSERM, CNRS, ESPCI Paris, PSL Res. Univ., Paris, France; [5]BBE, Calif Inst. of Technol., Pasadena, CA

Abstract: Brain-machine interfaces (BMIs) directly link the brain to external devices. They already enable users with chronic paralysis to control neuroprosthetics, including robotic arms and computer cursors. They promise future applications in smart home control, speech prosthetics, and closed-loop neurostimulation. However, the most advanced BMIs are still highly invasive and typically require re-training or re-calibration between subsequent sessions and different users. Recently, functional ultrasound imaging (fUSI) was introduced as a revolutionary hemodynamic technique that is minimally invasive (i.e. epidural) and portable while maintaining excellent spatiotemporal resolution (100 micron, up to 10 Hz) and a wide field of view (~2 cm). Previously, we showed that fUSI can be used to decode the neural correlates of movement planning in non-human primates (NHPs) - including timing, direction, and effector - before movement was executed and with single-trial sensitivity (Norman et al., *Neuron*, 2021). These results represented the first steps toward ultrasound-based BMIs that are less invasive, high resolution, and scalable. However, previous fUSI movement decoding models still required subject and session-specific re-training. Although fUSI imaging data are visually similar between NHPs and recording sessions, small movements and deformation of the brain parenchyma limit the performance of univariate decoding techniques that rely on pixel-wise changes in activity. Here, we present “fUSInet”, a convolutional neural network (CNN) that extends the capabilities of fUS-BMIs using a single model for generalized decoding between two NHPs and across recording sessions spanning 45 months. fUSInet recognizes the location and activity of cortical regions in NHP posterior parietal cortex (PPC) relative to reliable anatomical landmarks (e.g. intraparietal sulcus). It is therefore robust to animal- and session-specific changes in the precise

location of regions with specialized motor function, e.g. spatial planning. Thanks to this attribute, fUSInet outperforms previous fUSI models in decoding movement direction and effector within a single subject and day. In addition, performance is sustained when training on a corpus of fUSI data from one subject to classify novel data from a second subject or from subsequent scanning sessions, i.e. days. These results represent an important step toward BMIs that are less invasive and generalizable to naïve subjects and require less frequent re-training.

Disclosures: S.L. Norman: None. **W.S. Griggs:** None. **C. Rabut:** None. **D. Maresca:** None. **V.N. Christopoulos:** None. **C. Demené:** None. **M. Tanter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus. **M.G. Shapiro:** None. **R.A. Andersen:** None.

Poster

P551. Neurophysiology: Decoding and Neural Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P551.08

Topic: E.04. Voluntary Movements

Support: NIH National Institute on Deafness and Other Communication (R01DC008358)
Office of Naval Research, Pew Latin American Fellowship in the Biomedical Sciences (E.A.)
IIE Fulbright & Obra Social La Caixa (P.M.T)

Title: Neural population dynamics during vocal behavior

Authors: *P. M. TOSTADO[1], E. M. ARNEODO[2], T. GENTNER[2], V. GILJA[3];
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Abstract: The increasing capacity of neuroscientists to record the electrophysiological activity of large numbers of individual neurons permits the possibility of understanding brain dynamics that may appear imperceptible in individual unit responses. This is of particular interest when studying complex natural behaviors, like vocalization. To better understand the neural mechanisms that enable complex motor-vocal behavior, we recorded the simultaneous activity of 145 single neurons in the robust nucleus of the arcopallium (RA), a forebrain motor region, of male zebra finches engaged in unconstrained vocal behavior. We apply latent factor-based models to gain insight into the temporal dynamics of neural populations that drive vocal production. The characteristic stereotypy of zebra finch songs allows us to record high numbers of unconstrained, yet highly similar vocalizations in free-behaving birds. We find that the spiking activity across populations of individual RA neurons is well described by a pattern of smooth trajectories in the latent neural space during stereotyped singing (Figure 1). Moreover, the dynamics of the low-dimensional state-space of RA neural trajectories track single notes

categorically and reflect more subtle variability across different renditions of the bird's song. Similar observations are reported in populations of cortical neurons of the primary motor cortex (M1) of macaques during movement generation (Gallego et al. 2018). Our results point to common principles that may underlie the encoding of complex natural motor actions in specific brain regions or circuits.

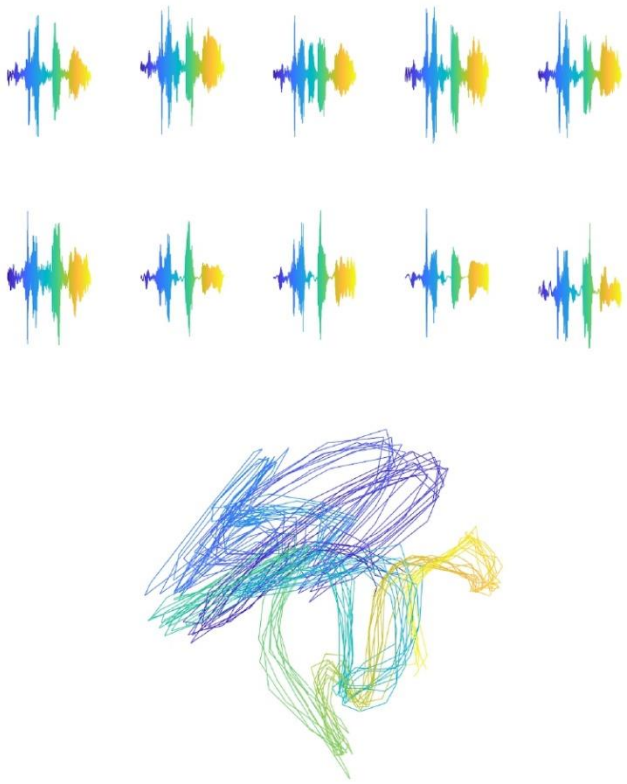


Figure 1. Ten examples of the raw audio waveforms of recorded vocal renditions of zebra finch song (top). Corresponding neural trajectories extracted from the population activity of RA 145 neurons and projected on a 3D latent neural space (bottom).

Disclosures: P.M. Tostado: None. **E.M. Arneodo:** None. **T. Gentner:** None. **V. Gilja:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.01

Topic: F.01. Neuroethology

Support: DFG 233886668 / GRK1960
Graduate School for Biological Sciences University of Cologne

Title: Morphology, Biomechanics, and Function of Leg Campaniform Sensilla in Drosophila melanogaster

Authors: *G. DINGES[1], K. FENG[2], T. BOCKEMÜHL[1], A. BLANKE[3], A. BÜSCHGES[1]; [1]Univ. of Cologne, Cologne, Germany; [2]Queensland Brain Institute, Univ. of Queensland, St Lucia, Australia; [3]Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

Abstract: Locomotion is a crucial component of animal survival that must be adaptable to be consistently successful. This adaptability is necessary for walking insects to overcome complex and changing terrains. During walking, sensory organs monitor motor output and provide dynamic feedback, both modifying and reinforcing, onto central network components. Campaniform sensilla (CS), cuticular sensory organs found on the majority of insect limb segments, encode highly dynamic strains that occur in the cuticle. In *Drosophila melanogaster*, leg CS research remains limited, and it is unclear how different CS and whole CS fields encode specific strains or which are necessary and sufficient to alter motor programs. The genetic tractability of *D. melanogaster* provides the opportunity to study these organs and their complex network interactions without the limitation of only using mechanical manipulations. To understand the interplay of morphology and function we utilized high-resolution imaging and optogenetic manipulations to investigate CS function in *D. melanogaster*. We targeted the outer morphology of CS using electron microscopy and thereby demonstrated interindividual and interleg variability in CS number and positions. Further, we used nano-computed tomography to investigate complex morphological CS field arrangements. This underlined that CS substructures unite individual CS within a field and might alter both the magnitude and direction of strains. To then correlate CS morphology with function, we tested how CS subsets influence leg movements and coordination using transient optogenetic manipulations in intact walking flies. This demonstrated the involvement of small CS subsets in two leg CS fields for these behaviors, highlighting their importance for both kinematic and temporal coordination. Inhibiting varying CS subsets leads to alterations in interleg coordination, while activation of the same subsets was sufficient for eliciting limb movements that changed joint angles. Interestingly, the effects of both inhibition and activation show that different leg types respond differently to CS feedback. Connecting CS morphology in the context of species-specific biomechanics with behavior

requires experiments investigating each of these aspects as part of a holistic system. Our work underlines the diversity in leg CS morphologies, how this diversity can affect field biomechanics, and the function of different constellations of CS in walking and postural control.

Disclosures: G. Dinges: None. **K. Feng:** None. **T. Bockemühl:** None. **A. Blanke:** None. **A. Büschges:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.02

Topic: F.01. Neuroethology

Title: Flexible, adaptive control of escape behaviour in mice

Authors: *S. C. LENZI, L. COSSELL, B. GRAINGER, S. F. OLESEN, T. BRANCO, T. W. MARGRIE;
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Abstract: Innate behaviours are a powerful model for understanding how sensory information is used by the brain to select behaviour. Predator avoidance behaviours such as freezing and escape are of particular interest since these stimulus-evoked responses represent behavioural manifestations of a dynamic decision-making process that is fundamental to survival (Evans et al., 2018). Over the lifespan of an individual, however, the threat value of particular agents in the environment undergoes constant revision (Cooper & Blumstein, 2015) and repeatedly escaping to a particular stimulus may no longer be the optimal behavioural strategy (Rossier et al., 2020). This form of cognitive control and learning highlights not only the flexibility of innate behaviour in general (Tomsic et al., 2017), but presents an elegant model for understanding how environmental experience influences behavioural choice at the level of neuronal circuits. To begin to understand what ethological factors shape this type of adaptive control we devised an experimental paradigm to probe the key determinants of escape behaviour and its learned suppression in the laboratory mouse, *Mus musculus*. First, we found that robust escape to visual looming stimuli is strongly influenced by factors such as social context, but not environmental enrichment. Second, we found that mice can rapidly learn to suppress escape to looming stimuli and that its abolishment can persist for weeks. This learned suppression of innate escape (LSIE) is not a generic adaptation to threat stimuli since flight responses to novel live prey and simulated auditory threats presented in the same arena location are maintained. Finally, we found that a critical determinant for the expression of LSIE is the temporal history of threat escape, suggesting that the underlying process is not simply desensitisation to repeated presentation of threat stimuli. Therefore, multiple ethological factors shape threat avoidance in mice. This rich

behavioural plasticity together with tractable genetics can be exploited to understand how neuronal circuit plasticity gives rise to flexible decision-making.

Disclosures: S.C. Lenzi: None. **L. Cossell:** None. **B. Grainger:** None. **S.F. Olesen:** None. **T. Branco:** None. **T.W. Margrie:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.03

Topic: F.01. Neuroethology

Support: NIH/NIDCR R00-DE02680

Title: Identification of pain hypersensitivity phenotypes across genetically diverse mouse lines.

Authors: *J. BURDGE, I. ABDUS-SABOOR;
Columbia Univ., New York, NY

Abstract: Identification of pain hypersensitivity phenotypes across genetically diverse mouse lines. Justin Burdge[1], Ishmail Abdus-Saboor[1].[1] Columbia University
The same pain can cause wildly different reactions depending on who experiences it. The key to understanding why lies in the genetics that underlie the pain pathway. Pain genes have led to the identification of multiple components of the peripheral pain way and how they lead to the sensing of painful stimuli and could in the same way be used to elucidate the pain pathway in the central nervous system (CNS). Identifying these central pain genes is essential to understanding the neural pathways that the brain uses to construct the sensation of pain and execute pain behaviors in response to noxious stimuli. Here we use the model of thermal escape jump, a behavior that originates from the brain, to examine how genetic variation leads to differences in pain behavior independent of pain sensitivity. We tested 18 genetically diverse inbred mouse lines to determine the levels of escape jump behavior they showed when place on a hot plate at either extreme hot or cold temperatures, and then tested them for thermal sensitivity to heat and cold for comparison. Our results found that C57/BL6 mice, which are commonly used in pain research, are an outlier in terms of thermal escape jump behavior when compared to non C57 strains. Comparisons with several strains showed decreases in this behavior which could not be explained by differences in thermal sensitivity. This testing found heightened jumping behavior without any significant difference in thermal sensitivity, with C57BL/10SnJ mice, a subline of C57 mice, showing more than 10 times the jumping behavior exhibited by C57/BL6 mice. Further when tested for general escape behavior using the tail suspension assay C57/10SnJ mice showed twice the escape behavior as C57BL/6J mice. Critically, these findings indicate that these differences in jumping behavior are not caused by changes in nociception, resulting from

differences in the periphery, but instead to the behavioral response itself which could only result from changes in the CNS. These results establish C57BL/10SnJ mice as targets for genetic analysis through which the specific neural circuitry underlying behavioral reactions to pain could be established.

Disclosures: J. Burdge: None. **I. Abdus-Saboor:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.04

Topic: F.01. Neuroethology

Support: NSF # 11520-02-412009377

Title: Investigating a potential magnetocaloric mechanism for magnetic field transduction by C. elegans.

Authors: *T. AWE[1], C. BAINBRIDGE[1,2], P. FREEBAIRN[1], A. G. VIDAL-GADEA[1]; [1]Sch. of Biol. Sci., Illinois State Univ., Normal, IL; [2]Univ. of Rochester Med. Ctr., New York, NY

Abstract: The magnetic field of the earth is used in navigation by many species across taxa. While the list of species able to navigate their environment with the use of the earth's magnetic field continues to grow, little is understood about the molecular and cellular underpinnings of this behavior. Two main mechanisms have been proposed for magnetic field transduction. The light-dependent mechanism is hypothesized to involve cryptochromes working in the retina of some species to generate magnetosensitive radical pairs in the presence of light. The second, more prevalent, mechanism hypothesized involves the presence of magnetic particles coupled to stretch receptors in the tissues of many magnetosensitive species: the magnetomechanic mechanism. Recently, Bell et al (2019) proposed an alternative rotating magnetocaloric transduction mechanism involving the detection of heat generated by magnetic nanoparticles as they move through the earth's magnetic field. We previously showed that the nematode *C. elegans* orients to magnetic fields in a light-independent manner. Detection of magnetic fields by *C. elegans* is mediated by a pair of polymodal sensory neurons, AFDs. These neurons are the primary thermosensory neurons in *C. elegans* and respond to minute changes in temperature. We are working to establish the mechanism for magnetic transduction in *C. elegans*, performing experiments designed to test the potential use of magnetocaloric and magnetomechanic transduction mechanisms. To this end, we exposed worms to either strong magnetic pulses, or to oscillating magnetic fields, and testes their ability to orient to magnetic fields. Our current findings favor a magnetocaloric mechanism for magnetic orientation in worms. *C. elegans* is an

excellent system for studying the molecular, cellular, and behavioral basis of magnetic field detection.

Disclosures: T. Awe: A. Employment/Salary (full or part-time):; Illinois State University. **C. Bainbridge:** None. **P. Freebairn:** None. **A.G. Vidal-Gadea:** A. Employment/Salary (full or part-time):; Illinois State University.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.05

Topic: F.01. Neuroethology

Support: Kavli Neural Systems Institute Pilot Grant
Kavli Neural Systems Institute Postdoctoral Grant
Howard Hughes Medical Institute Investigator Support

Title: Mosquito host seeking is a persistent state for temporal multimodal integration

Authors: *T. SORRELLS, A. PANDEY, A. ROSAS, L. VOSSHALL;
Rockefeller Univ., New York, NY

Abstract: Female *Aedes aegypti* mosquitoes seek out humans to bite them and obtain a meal of blood. To perform this task, they require sensory cues from multiple different modalities to bite at high rates. Although this task can take minutes, how long the behavior is sustained and whether host cues are integrated over time is unknown. We used optogenetic tools and machine learning-based classification of behavior to investigate how mosquitoes respond to multimodal host cues. We found that the host cue carbon dioxide induces a persistent internal state change that outlasts the stimulus by several orders of magnitude. This host-seeking state is characterized by increased search behaviors and altered responses to subsequent heat and taste stimuli. This state is only found in host-seeking female mosquitoes; other sensory stimuli tested did not induce persistent responses. Furthermore, we found that the gene *fruitless*, which controls persistent social behaviors in other insects, controls the persistence but not the initial carbon dioxide response in mosquitoes. This suggests that the *fruitless* functions of sexual dimorphism and persistent activity were co-opted during the evolution of mosquito feeding behavior. Our results demonstrate that mosquitoes integrate multisensory information about their human hosts over minutes through a persistent internal state.

Disclosures: T. Sorrells: None. **A. Pandey:** None. **A. Rosas:** None. **L. Vosshall:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.06

Topic: F.01. Neuroethology

Title: The neural circuit underlying light-avoidance behavior in Drosophila larvae

Authors: *D. SAVAS[1], A. SORKAÇ[2], Y. SAVVA[3], M. TALAY[4], G. BARNEA[2];
[2]Neurosci., [1]Brown Univ., Providence, RI; [3]Shape Therapeutics, Inc., Seattle, WA; [4]Mol. and Cell. Biol., Harvard Univ., Boston, MA

Abstract: Sensory systems collect information about the environment and employ neural circuits to process this information to elicit appropriate behavioral responses and maximize survival of the organism. To understand how these circuits function, a combined approach including anatomical and behavioral analyses is required. *Drosophila melanogaster* is a great model to study such circuits due to the vast availability of resources and tools and its ease of use. The larvae of *Drosophila melanogaster* exhibit a robust light-avoidance behavior, also known as photophobia. Previous studies identified two distinct types of neurons that are necessary for photophobic behavior: the light detecting Rh5 photoreceptor in the larval visual organ and the prothoracicotropic hormone (PTTH)-expressing neurons in the central brain. However, how information from the Rh5 photoreceptor neurons is conveyed to the PTTH neurons remains unknown. To examine this, we modified the circuit tracing tool *trans*-Tango, such that it would be suitable for tracing circuits in fly larvae. We termed this version *trans*-Tango MkII. Our anatomical analysis revealed that Rh5 and PTTH neurons are not directly connected. Rather, they are connected through two types of clock neurons: Rh5 photoreceptor neurons synapse onto the 5^{th} Lateral Neuron (5^{th} LaN), which then synapses onto one of the two pairs of dorsal neurons, DN2s. The DN2s, in turn, synapse on the PTTH neurons allowing for a linear information flow from the Rh5 photoreceptors to the PTTH neurons. We complemented these anatomical studies by functional analysis whereby we combined selective inhibition of the various types of clock neurons in behavioral assays. This analysis revealed that the 5th LaN and DN2s are necessary for the larval photophobic behavior since inhibition of these neurons yielded lower levels of photophobia compared to genetic controls. We next aimed to examine whether the activation of the 5^{th} LaN and DN2s is sufficient to elicit photophobia. To this end, we used selective optogenetic activations of either of these neurons in Rh5 photoreceptor mutant larvae that are deficient in photophobic behavior. This analysis revealed that indeed activation of the 5^{th} LaN and DN2s rescues photophobic behavior, whereas environmental and genetic controls do not. As a control, we exposed the Rh5 mutant animals to the light source used for optogenetic activation and they did not show any aversion or attraction to the stimulus. Our study revealed a linear neural circuit consisting of four layers that underlies the photophobic behavior in *Drosophila* larvae.

Disclosures: D. Savas: None. **A. Sorkaç:** None. **Y. Savva:** None. **M. Talay:** None. **G. Barnea:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.07

Topic: F.01. Neuroethology

Support: NIH K99NS119749

Title: Functional modules within a distributed neural network control feeding in a model medusa

Authors: *B. WEISSBOURD[1], T. MOMOSE[2], A. NAIR[1], A. KENNEDY[1,3], B. HUNT[1], D. ANDERSON[1];
[1]Caltech, Pasadena, CA; [2]Sorbonne Université, CNRS, Lab. de Biologie du Développement de Villefranche-sur-mer (LBDV), Villefranche-sur-mer, France; [3]Dept. of Physiology, Feinberg Sch. of Med. Northwestern Univ., Chicago, IL

Abstract: Jellyfish are free-swimming, radially symmetric organisms with complex behaviors that arise from coordinated interactions between distinct, autonomously functioning body parts. This behavioral complexity evolved without a corresponding cephalization of the nervous system. The systems-level neural mechanisms through which such decentralized control is achieved remain unclear. Here, we address this question using the jellyfish, *Clytia*, and present it as a new neuroscience model. We describe a coordinated, asymmetric behavior in which food is passed from the umbrellar margin to the central mouth via directed margin folding. Using newly developed transgenic jellyfish lines to ablate or image specific neuronal subpopulations, we find, unexpectedly, that margin folding reflects the local activation of neural subnetworks that tile the umbrella. Modeling suggests that this structured ensemble activity emerges from sparse, local connectivity rules. These findings reveal how an organismal behavior can emerge from local interactions and functional modularity in the absence of a central brain.

Disclosures: B. Weissbourd: None. **T. Momose:** None. **A. Nair:** None. **A. Kennedy:** None. **B. Hunt:** None. **D. Anderson:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.08

Topic: F.01. Neuroethology

Support: R56 NS110951

Title: Epigenetic regulation of developmental song learning in juvenile zebra finch

Authors: *G. KUNZELMAN[1], S. E. LONDON[2];
[2]Dept of Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: Neural properties that regulate developmental learning are particularly difficult to define because both maturation and experience can influence number of cells, cell subtypes, cellular functionality, and synaptic connections, all features that influence learning and memory. The complement of protein coding and noncoding RNAs transcribed from the genome largely determines these cellular properties. Transcription is regulated by transcription factors (TF), proteins that bind "regulatory" genomic regions at transcription factor binding sites (TFBSs). Epigenetic modifications, including those on histone proteins, modulate TFBS accessibility. H3K27ac is a histone modification that reliably denotes accessible TFBSs. To empirically determine the neural properties that support learning of complex behavior, we paired chromatin immunoprecipitation for H3K27ac and high-throughput DNA sequencing (ChIP-seq), and a well-known developmental learning paradigm in zebra finches. We assayed the auditory forebrain, a region required for sensory song learning. We bioinformatically identified differentially accessible genomic regulatory regions and TFBSs in juveniles who differed based on age, sex, and prior song experience, factors that are central to the ability to learn song during development. Based on the differential peak numbers, age has the most robust effect on the H3K27ac-associated regulatory regions. In males, the biggest difference was between two ages when sensory song learning is robust. The TFBSs within these differential regions indicate that neurogenesis, gliogenesis, and neuronal differentiation are driving these developmental changes regulated by a combination of maturation and accumulated song experience. Interestingly, older, age-matched males that differed only in their prior exposure to tutor song were not as distinctly different, suggesting that the extent of changes in the H3K27ac-associated regulatory regions is largely set by maturation by this stage. We found the most robust sex differences in birds still capable of sensory song learning, and overrepresented TFBSs suggest that neuronal circuit organization is differentially regulated between males and females across maturation. This is interesting because both males and females form sensory song memories as juveniles but only males sing; these data suggest a sex difference in sensory song learning mechanisms. Together, our comprehensive evaluation of epigenetically-defined accessible regulatory regions allows for novel insight into the emergence of neural learning circuits in a brain area required for developmental learning of a complex behavior.

Disclosures: G. Kunzelman: None. **S.E. London:** None.

Poster

P582. Sensory Motor Systems IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P582.09

Topic: F.01. Neuroethology

Support: NIH Grant K01 AG057833

Title: Chemotaxis in a neuromechanical model of C. elegans

Authors: M. IVANITSKIY, V. BOOTH, *E. GOURGOU;
Univ. of Michigan, Ann Arbor, MI

Abstract: *C. elegans* nematodes exhibit a wide variety of behaviors, including chemotaxis and associative learning, with only a very limited amount of neural circuitry. Although the connectome of *C. elegans* is mapped, the weights of the connections are not, making the modelling of behavior an open problem. Here we build on our previous work and extend a neuromechanical model for *C. elegans* locomotion to display chemotaxis mediated by a single chemosensory neuron. Chemotaxis is achieved through incorporating the chemosensory neuron AWA, as well as the interneurons AIY and RIA, the latter having bidirectional connections to proprioceptive motor neurons. We use an evolutionary algorithm to optimize the model parameters to exhibit chemotaxis via locomotion towards a food source. We find that simply extending the neural model of chemotaxis in a point worm to the neuromechanical model results in a poorly performing model worm. The suggested model, with additional network complexity due to the AIY and RIA interneurons, and their bidirectional connections to SMDD and SMDV, is found to perform more realistically. We postulate that the RIA-SMDx reciprocal connections, suggested in the literature, allow the chemosensory control circuit to compensate for varying proprioceptive feedback introduced by navigation towards a food source. We are working on introducing learning in the form of synaptic plasticity, to account for known cases of food-triggered associative learning. We envision this work to provide the framework for modeling and understanding more complex, whole-body *C. elegans* behaviors.

Disclosures: M. Ivanitskiy: None. **V. Booth:** None. **E. Gourgou:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.01

Topic: F.01. Neuroethology

Support: NIH F31 HD098772-02
NIH 5R01NS099288-05

Title: Models of juvenile zebra finch song changes using data-driven acoustic features

Authors: *S. BRUDNER[1], J. GOFFINET[1], J. M. PEARSON[2], R. D. MOONEY[3];
[2]Biostatistics and Bioinformatics, [1]Duke Univ., Durham, NC; [3]Duke Univ. Dept. of Neurobio., Durham, NC

Abstract: Song copying by juvenile zebra finches has emerged as a powerful laboratory model for aspects of imitative learning, including human speech learning. However vocal behavior data is high-dimensional and complex, qualities that introduce analysis challenges. Efforts have been made to improve the tractability of vocal behavior analysis, recently including the use by our group and others of variational autoencoders (VAEs), an unsupervised dimensionality reduction technique. Here we extend previous work on VAE-based vocal features to the analysis of vocal changes during zebra finch development. In particular, we develop two approaches: (1) in song learning "forward models," we explicitly model the dependence of syllable acoustic distributions on animal age by developing age-dependent multivariate gaussian distributions in VAE latent space; (2) in song learning "reverse models," we predict animal age from vocal features, enabling us to assess the maturity of individual song renditions. We show that our models reproduce state-of-the-art results about circadian rhythms in syllable maturation. Our models also reveal circadian changes in rendition-to-rendition syllable variability, consistent with a prior report, while also allowing us to explore previously inaccessible aspects of this variability. Finally, we use these methods to show that a variety of circadian behavioral features associated with learning to copy a tutor's model song also occur in the behavior of isolate juveniles without an appropriate tutor model.

Disclosures: S. Brudner: None. **J. Goffinet:** None. **J.M. Pearson:** None. **R.D. Mooney:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.02

Topic: F.01. Neuroethology

Support: NSF Award EF-1822476

Title: Recognition of birdsong syllables with ultra-sparse spike sequence representations

Authors: *L. E. S. TAVARES, D. Z. JIN;
Deptartment of Physics, Pennsylvania State Univ., University Park, PA

Abstract: Birdsong is important for research in vocal learning and communication in animals. For many research questions, accurate annotations of individual vocal elements are indispensable. However, manually annotating large sets of songs can be cumbersome and expensive. While tools for automatic recognition of syllables exist, they either require large amounts of pre-annotated data or have sub-optimal accuracy. Such limitations discourage adoption of current automatic tools in research.
Here, we present a method for automatic syllable recognition that requires only a few training exemplars and achieves error rates low enough for practical applications. As new annotations become available, the system's accuracy can be continuously improved. We show results for a number of songbirds commonly used in neuroscience experiments.
Our method represents vocal objects as sequences of overlapping acoustic events. Groups of feature-detecting neurons encode the onsets and offsets of these events into ultra-sparse sequences of spikes. Continuous recognition is done by a template-matching scheme, which compares the relative delays of the spikes to stored templates and integrates them over time. To ensure that all acoustic events are detected, including those eliciting weak responses, we also adopt a threshold adaptation mechanism, which modifies the neuron's receptive field and makes it less selective. In a second stage, a layer of specialized binary decision making units selects a single prediction for each syllable in the song. This prevents insertions arising from the adaptation mechanism. The final output is a list of syllable onset times, offset times and labels. We demonstrate our results for Bengalese finches, zebra finches and canaries. The phrase structure of the canary song allows us to further improve the recognition results by predicting entire phrases rather than individual syllables within them. We show that, for most birds, the error rate can be as low as 5% with less than 10s of training data, outperforming existing deep learning tools for continuous syllable recognition. In addition, the error decreases as more data becomes available for training.

Disclosures: L.E.S. Tavares: None. **D.Z. Jin:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.03

Topic: F.01. Neuroethology

Title: Segmenting vocalizations for downstream analysis with a deep neural network, TweetyNet

Authors: *D. NICHOLSON;
Emory Univ., Washington, DC

Abstract: Many analyses of experiments studying vocal learning and control require time-consuming annotation of the individual elements of animal vocalizations. For example, many

researchers studying birdsong annotate a syllable of interest manually, with a GUI. Other methods for analyzing vocal behavior do not require annotation of individual elements. Instead they require segmentation of audio into two categories: vocalizations and non-vocalizations. All of the segments labeled as "vocalization" are then passed to a downstream algorithm, for example those that compute measures of similarity. It remains unclear how reliable and robust different algorithms for segmentation are. To further understand this, here we test a deep neural network architecture we developed, TweetyNet (https://github.com/yardencsGitHub/tweetynet), as a "front end" for segmenting audio into vocalizations that are then passed to downstream algorithms for further analysis. Previously we demonstrated that TweetyNet automates detailed syllable-level annotation of very large datasets of Bengalese Finch and canary song (https://www.biorxiv.org/content/10.1101/2020.08.28.272088v2). Here we show that TweetyNet performs well as a simple binary classifier, dividing audio into vocalization and no-vocalization segments. TweetyNet reliably recovers segments of zebra finch song (http://ofer.hunter.cuny.edu/songs) and bat vocalizations (https://www.nature.com/articles/sdata2017143), achieving near-perfect accuracy (~99% frame error rate across all animals). Experiments in progress test the robustness of this approach with simulated noisy data, and test application of TweetyNet to human speech. We will also give an update on development of our toolbox that makes it easier for researchers to apply neural networks models to the study of animal vocalizations (https://vak.readthedocs.io/en/latest/).

Disclosures: D. Nicholson: None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.04

Topic: F.01. Neuroethology

Support: NSF Award EF-1822476

Title: Estimating the Number of Hidden States in Birdsong Syntax

Authors: *J. LU, D. Z. JIN;
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Abstract: Syllable sequences in the songs of many songbird species such as the Bengalese finch and the canary are variable and complex. Such sequences follow long-range rules (Jin & Kozhevnikov 2011 and Cohen et al. 2020), and cannot be described by the basic Markov model, where each syllable is represented by only one state. Instead, the long-range rules need to be captured with several states representing the same syllable. With such a “many-to-one” mapping from the states to the syllables, the statistical state transition models become the Partially

Observed Markov Model (POMM) (Jin & Kozhevnikov 2011 and Jin 2009). However, it is unclear how to estimate the number of states for each syllable from the observed syllable sequences. Here we propose a efficient algorithm to solve the problem. The key is to construct the moment matrix. For each syllable, we find the past subsequence of length N preceding the syllable, and the future subsequence of length N following the syllable. The number of transitions from each past subsequence to each future subsequence is counted and normalized so that the sum is equal to 1. These numbers become entries to the moment matrix for the syllable. We then use Singular Vector Decomposition (SVD) to find the optimal low rank approximation to the moment matrix. The approximation is statistically validated by finding the p-value through the bootstrap resampling of the sequences. The estimated rank is the number of the hidden states for the syllable. When the underlying process is POMM, our algorithm returns a lower bound that is necessary to describe the song syntax given the finite data. In tests with birdsong-like random POMMs, we show that the lower bound approach the true number of hidden states as the number of observed sequences increases. Our algorithm also provides indications when the syllable sequences are not describable with POMM. A simple example of such sequences is A^nB^n, where n copies of syllable A's are followed by exactly n copies of syllable B's (Gentner et al. 2006). We find that as the number of observed sequences increases, the number of states for each syllable keeps increasing with no sign of converging to finite values. This is indicative of sequences beyond the capabilities of POMM. We show how our algorithm provides insights into interesting problems such as the learning of song syntax in the Bengalese finch (James et al. 2020).

Disclosures: J. Lu: None. **D.Z. Jin:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.05

Topic: F.01. Neuroethology

Support: NSF Grant DGE1255832
NSF Award EF-1822476

Title: Analysis of budgerigar vocalizations

Authors: *A. R. ZENDER[1], L. ACKERT-SMITH[2], M. A. LONG[2], D. Z. JIN[1];
[1]Dept. of Physics, Pennsylvania State Univ., University Park, PA; [2]Neurosci. Inst., NYU Sch. of Med., New York, NY

Abstract: Parrots are capable of an astounding degree of vocal learning and rich social interactions, making them a potentially important model system for understanding the neural

basis of vocal communication. However, the interactive vocal repertoire of the parrot remains poorly understood. Here we use advanced methods to study vocal exchanges in the budgerigar, a small Australian parrot. Pairs of birds were placed in an arena with 2-4 microphones and 4 cameras for continuous recordings lasting several months. We observed the vocalizations of the birds while they were together as well as while they were separated by various partitions designed to selectively impair visual and/or tactile access. We developed a range of computational tools to analyze their vocalizations including contact calls, alarm calls, and warble. Audio files were segmented into potential vocalizations using the wave envelope, and nine acoustic features were computed; the median values of each resulted in 9-dimensional vectors corresponding to single vocal elements. A support vector machine (SVM) was then employed to group the elements into contact calls, alarm calls, warble, and various sources of environmental noise. To optimize the performance of the SVM, we used a greedy search to show that the best accuracy occurred when using just five of the acoustic features, yielding an error of 7.3 percent on our data set compared with hand labeled designations. We used these classifications to examine the impact of the partition conditions on vocal use. In initial observations, we found that both visual and tactile occlusion led to a significant change in vocal behavior, suggesting that multimodal sensory interaction may be an integral part of parrot communication.

Disclosures: A.R. Zender: None. **L. Ackert-Smith:** None. **M.A. Long:** None. **D.Z. Jin:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.06

Topic: F.01. Neuroethology

Support: NSF DGE-1444932
NIH R01NS084844
NIH R01NS099375
Simons-Emory International Consortium on Motor Control

Title: Sequence-dependent modulation of neural activity in the songbird basal ganglia

Authors: *A. WOOD, R. CHHIBBER, S. J. SOBER;
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Abstract: Skilled motor behaviors such as speaking, writing, or playing the piano require a combination of fine control of moment-to-moment movement kinematics and flexible sequencing of longer behavioral “motifs”. The basal ganglia are a group of subcortical nuclei implicated in a wide range of motor and cognitive functions, including motor kinematics and

motor sequencing. The songbird Bengalese finch (*Lonchura striata*) has a song-specialized basal ganglia nucleus (Area X) which has previously been implicated in both kinematic and sequential modulation of song. Area X contains neurons that are homologous to mammalian striatal and pallidal neurons. Using *in vivo* electrophysiology in adult male Bengalese finches, we first show that classes of Area X striatal and pallidal neurons can be separated by firing properties. We hypothesized that individual Area X pallidal neuron activity reflects both phonological and sequential information. To test this, we assess the tuning of individual neurons by analyzing activity at points in song where syllable sequencing is variable using a bootstrap analysis of premotor firing rates and linear correlations. Our preliminary data display strong tuning for sequence but an apparent lack of tuning for phonological structure, consistent with reinforcement learning models of song learning which predict that Area X integrates dopaminergic reward signals with contextual information about recent motor commands.

Disclosures: A. Wood: None. **R. Chhibber:** None. **S.J. Sober:** None.

Poster

P585. Vocal/Social Communication: Avian III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P585.07

Topic: F.01. Neuroethology

Support: NIH R01NS084844
NIH R01EB022872

Title: Shared neural circuit mechanisms for auditory and non-auditory guided vocal learning in adult songbirds

Authors: *J. MCGREGOR[1], A. GRASSLER[1], P. I. JAFFE[3], A. L. JACOB[2], M. S. BRAINARD[4], S. J. SOBER[2];
[2]Biol., [1]Emory Univ., Atlanta, GA; [3]Neurosci. Grad. Program, UCSF, San Francisco, CA; [4]Dept Physiol., UCSF Ctr. For Integrative Neurosci, San Francisco, CA

Abstract: Songbirds and humans share the ability to use sensory feedback to guide the learning of their vocal output, and both species have evolved unique neural circuitry that is specialized for promoting vocal learning. Auditory feedback is particularly important for shaping vocal output, and numerous prior studies have revealed the neural mechanisms underlying auditory-guided vocal learning. Non-auditory signals can also shape vocal output. However, the neural circuit mechanisms that underlie non-auditory vocal learning are unclear. Here, we assessed whether and how non-auditory feedback guides vocal learning in adult songbirds. First, we tested whether non-auditory feedback cues (cutaneous electric shocks) are sufficient to drive adaptive changes in vocal output. Next, we assessed the role of LMAN, the output nucleus of a specialized vocal

learning neural circuit, in this novel form of vocal learning. Finally, we evaluated the necessity of dopaminergic input to Area X, the basal ganglia nucleus of this vocal learning circuit, in non-auditory vocal learning. We found that this song learning circuit and its dopaminergic inputs are necessary for non-auditory vocal learning, demonstrating that this neural pathway is not specialized exclusively for processing auditory feedback to reshape vocal behavior. The ability of the songbird vocal learning pathway to process both auditory and non-auditory information to guide changes to vocal output may reflect a general principle for the neural systems that have evolved to support vocal learning across species.

Disclosures: J. McGregor: None. **A. Grassler:** None. **P.I. Jaffe:** None. **A.L. Jacob:** None. **M.S. Brainard:** None. **S.J. Sober:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.01

Topic: F.04. Stress and the Brain

Support: NIMH R01MH062044
2CI Neurogenomics

Title: Behavioral and physiological responses to social defeat stress and to BDNF-active drugs vary over the light-dark cycle

Authors: *E. SHAUGHNESSY, M. G. WELCH, A. M. ROSENHAUER, K. MALHAM, K. L. HUHMAN;
Georgia State Univ., Atlanta, GA

Abstract: Social stress is a salient risk factor for neuropsychiatric illnesses including major depressive disorder, post-traumatic stress disorder, and other anxiety disorders. Brain-derived neurotrophic factor (BDNF) has been implicated in the pathophysiology and treatment of these disorders, but there are conflicting data regarding the potential therapeutic use of BDNF-related manipulations in treating them. Our lab has shown that administration of an agonist (7,8-DHF) for the BDNF cognate receptor Tropomyosin receptor kinase B reduces the behavioral response to social defeat stress (Rosenhauer et al 2019). Conversely, Berton et al. (2006) showed that BDNF signaling is necessary for the behavioral response to social defeat stress. One difference between the studies is that the former ran behavior during the dark (active) phase of the daily light-dark cycle, whereas the latter studies were run during the light (inactive) phase. The purpose of this study was to determine whether responses to social defeat and to BDNF vary over the light-dark cycle and, if so, what brain regions are likely involved. In the first experiment, male (n=25) hamsters were defeated or experienced the no defeat protocol in the

light or the dark phase of the daily cycle, given 7,8-DHF (5mg/kg in 50% DMSO 50% saline) or vehicle, and tested for conditioned defeat 24 hours later. Administration of 7,8-DHF decreased social avoidance when defeat, treatment, and testing occurred during the dark phase. Conversely, animals that were defeated, received 7,8-DHF, and were tested in the light showed an increase in social avoidance. Next, we examined neural activation of selected brain regions in the putative neural circuit that controls responses to social defeat stress in male (n=28) and female (n=25) Syrian hamsters using the immediate early gene product C-Fos as a marker. As in Experiment 1, hamsters were defeated or not during the dark or the light phase and immediately given 7,8-DHF or vehicle. 90 minutes later, brains were collected and processed for C-Fos immunohistochemistry. There were significant main effects of sex, defeat, and 7,8-DHF in specific brain regions. In particular, there were significantly more C-Fos-positive cells in all regions in males than in females. There was a significant effect of defeat on C-Fos in the infralimbic (IL) cortex and anterior cingulate cortex (ACC), but this effect was observed only in hamsters defeated and tested during the dark phase but not during the light phase. Together, these data underscore the importance of considering lighting and circadian variables when assessing the therapeutic potential of new treatments for stress-related disorders.

Disclosures: E. Shaughnessy: None. **M.G. Welch:** None. **A.M. Rosenhauer:** None. **K. Malham:** None. **K.L. Huhman:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.02

Topic: F.04. Stress and the Brain

Support: NIAAA-R01-AA024095
Bowles Center for Alcohol Studies at the University of North Carolina at Chapel Hill

Title: (3α,5α)3-hydroxypregnan-20-one (3α,5α-THP) fails to attenuate stress-induced increases in serum corticosterone in male and female rats, but has sex and region specific effects on CRF expression

Authors: *G. BOERO, R. E. TYLER, C. A. TODD, T. K. O'BUCKLEY, I. BALAN, J. BESHEER, A. L. MORROW;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Neuroactive steroid (3α,5α)3-hydroxypregnan-20-one (3α,5α-THP, allopregnanolone) is the most potent positive modulator of $GABA_A$ receptors. It has previously been reported that 3α,5α-THP or 3α,5α-THDOC attenuate basal and stress-induced hypothalamic corticotropin-

releasing factor (CRF) mRNA expression as well as ACTH and corticosterone (CORT) serum levels in male rats (Patchev et al., 1994; 1996; Owens et al., 1992). We recently showed (Boero et al, 2021) that 3α,5α-THP regulation of basal levels of CRF are sex and region dependent. In this study, we investigated 3α,5α-THP regulation of stress-induced CRF across brain in male and female Sprague Dawley rats. Rats were injected with 3α,5α-THP (15mg/kg, IP) in hydroxypropyl-beta-cyclodextrin (45%) or an equivalent volume of vehicle and after 15 min subjected to a) 30 min of restraint stress (RS), sacrificed instantly or after 3hrs, or b) 10 min of swim stress (SS), return in their cage and sacrificed 10 min later. Brains and blood were harvested for western blot measurements of CRF and ELISA assay for CORT. Data were analyzed statistically by 2-WAY ANOVA followed by the Tukey's post-hoc test. In male rats, RS increased CRF levels 3hrs after the stress (Hyp +76%, $p<0.05$; CeA +74%, $p<0.05$; NAcc +176%, $p<0.05$; VTA +96%, $P<0.05$); no stress effect was detected in hippocampus. Serum CORT levels were elevated in male rats immediately after the RS (+495%, $p<0.0001$), and was still high 3hrs later (+272%, $p<0.05$). After SS, CORT levels increased as expected (+319%, $p<0.001$). 3α,5α-THP did not attenuate either stress response in male rats. In female rats, RS increased CRF levels immediately after stress, in hypothalamus (+100%, $p<0.01$) and hippocampus (+36%, $p<0.05$), and 3α,5α-THP attenuated the CRF stress response. No stress effect was detected in CeA, NAcc and VTA. Immediately after RS, serum CORT levels increased in female rats (+157%, $p<0.05$), but not 3hrs later. Moreover, SS induced a significant increase of serum CORT levels in female rats (+202%, $p<0.01$). 3α,5α-THP did not alter the CORT stress response after RS or SS. The data indicate that 3α,5α-THP only inhibits stress-induced CRF in hypothalamus and hippocampus of female rats. We previously showed (Boero et al., 2021) higher basal 3α,5α-THP levels in those brain areas of female vs male rats. Therefore higher basal levels, in addition to 3α,5α-THP administration, might attenuate the stress response in specific brain areas of female rats. Further studies will occur to clarify the role of 3α,5α-THP in the stress response.

Disclosures: G. Boero: None. **R.E. Tyler:** None. **C.A. Todd:** None. **T.K. O'Buckley:** None. **I. Balan:** None. **J. Besheer:** None. **A.L. Morrow:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.03

Topic: F.04. Stress and the Brain

Support: R03 DA051740-01

Title: Effects of chronic cannabis vapor self-administration on the neuroendocrine stress response in rats

Authors: *N. C. GLODOSKY[1], C. CUTTLER[1], T. G. FREELS[2], A. N. MALENA[1], H. R. WRIGHT[1], D. E. GINDER[1], H. V. WEIMAR[1], R. J. MCLAUGHLIN[1];
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Abstract: Stress is a significant risk factor for a multitude of mental, cardiovascular, metabolic, and immune diseases. Cannabis users report stress relief as their primary reason for use, although studies have found a positive relationship between cannabis use and the prevalence of stress-related disorders. Evidence from our lab indicates a blunted stress response in humans following chronic cannabis exposure. Specifically, we have shown that sober chronic cannabis users displayed a significantly smaller increase in cortisol following a stressor compared to non-using controls. However, these studies were unable to determine whether cannabis use directly *causes* alterations in the stress response, or whether an attenuated stress response represents a pre-existing risk factor for cannabis use. Additionally, it remains unclear what mechanisms are responsible for these neuroendocrine alterations in cannabis users.
Male and female Sprage Dawley rats (n=12/sex/group) were trained to self-administer vaporized cannabis containing 69.9% THC at one of four extract concentrations (0, 75, 150, or 300 mg/ml) daily for 30 days. Half of the rats were subjected to a 30 min acute restraint stress challenge 24 h after the final self-administration session while the other half served as no stress controls to assess stress reactivity. Given that corticotropin-releasing hormone (CRH) activity in the central nucleus of the amygdala (CeA) contributes to the emotional response to stressors, which is dampened in chronic cannabis users, we extracted brains from cannabis- and vehicle-exposed rats following acute stress (or no stress) exposure to assess CRH peptide content and CRH receptor type-1 (CRHR1) protein expression in the CeA under stress or non-stress conditions. Female rats earned significantly more vapor deliveries than male rats. Pre-vapor stress reactivity was not a predictor of self-administration rates in either sex. Importantly, cannabis self-administration reduced stress reactivity in female, but not male, rats specifically at the 150 mg/ml dose, which was also the concentration that was most readily self-administered in both sexes. Studies examining CRH and CRHR1 protein expression in the CeA are ongoing, but we predict that cannabis-exposed rats will have lower stress-induced CRH peptide content in the CeA compared to non-exposed rats.
These studies provide a foundation for understanding the consequences of chronic cannabis use and by using an animal model with strong internal and ecological validity, we are in an excellent position to identify causal effects of cannabis use and determine the neurobiological mechanisms by which they occur.

Disclosures: N.C. Glodosky: None. **C. Cuttler:** None. **T.G. Freels:** None. **A.N. Malena:** None. **H.R. Wright:** None. **D.E. Ginder:** None. **H.V. Weimar:** None. **R.J. McLaughlin:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.04

Topic: F.04. Stress and the Brain

Support: NIH Grant MH125306-01A1
NIH Grant MH104485-01A1

Title: Orexins through receptor subtypes (Orx_1 & Orx_2) differentially gate stress-prompted phenotype development

Authors: *J. YAEGER[1], T. R. SUMMERS[2], C. H. SUMMERS[2];
[2]Dept. of Biol., [1]Univ. of South Dakota, Vermillion, SD

Abstract: Stress-induced disorders, like anxiety, depression, and post-traumatic stress disorder (PTSD), are difficult to treat, in part, due to variability in both symptoms and treatment outcomes. These qualities, too, have made a mechanistic understanding of the pathophysiology associated with the disorders elusive and difficult to assess. As individuals react differently to stress, and therefore experience unique consequences of stress, it is sensible that an exploration of phenotype formation during periods of stress might uncover avenues toward treatment options for anxiety, depression, and/or PTSD. Using a preclinical assessment tool, the Stress Alternatives Model (SAM), a Go-No Go paradigm in which social stress promotes behavioral phenotype bifurcation (Escape/Resilient & Stay/Susceptible), we identified roles for the orexin system in regulating phenotype formation. Orexins (Orx_A & Orx_B) are hypothalamus-produced neuropeptides that regulate a range of behaviors, including arousal, feeding, motivation, and sleep. These neuromodulators have wide influence over several brain regions by targeting and activating two receptor subtypes (Orx_1 & Orx_2). In a series of experiments, we identified transcriptional relationships between orexin receptor genes in key brain areas, namely hippocampus, prefrontal cortex (PFC), and amygdala, and behavioral outcomes (like fear freezing) associated with phenotype development. In particular, in the basolateral amygdala (BLA), Orx_1 mRNA (*HCRTR1*) expression is positively correlated with freezing behavior in Escape mice, while Orx_2 (*HCRTR2*) is negatively associated with this response in Stay animals. Further, we revealed a role for the two orexin receptor subtypes in mediating stress behavioral responses in a counterbalanced fashion. Pharmacological inhibition (or genetic knockdown) of BLA Orx_1 promotes Escape/Resiliency, an effect that is mimicked with Orx_2 agonism. Alternatively, inhibition of Orx_2 and stimulation of Orx_1 prompts differential behaviors. Further, in the BLA Orx_1 antagonism has a dominant transcriptional effect on Stay animals causing increases in Orx_2, ERK_1, and BDNF expression. Contrastingly, Orx_2 inhibition promotes a reduction in Orx_2 and increases in $PLC_{\beta 1}$ and BDNF in Escape mice. Interestingly, orexin receptors in the BLA are mostly found on different cells with Orx_1 co-localizing predominantly with CamKIIα (a glutamatergic neuron marker) and Orx_2 being expressed more frequently with GAD67 (GABA neurons). Together, these results suggest Orx_1 and Orx_2 work together to balance stress responsivity.

Disclosures: J. Yaeger: None. **T.R. Summers:** None. **C.H. Summers:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.05

Topic: F.04. Stress and the Brain

Support: NIH Grant R15 MH125306-01A1
NIH Grant R15 MH104485-01A1

Title: Promotion of social stress in females reveals sex-independent viability of Orx_2agonist for the treatment of affective disorders

Authors: *C. H. SUMMERS[1], J. D. W. YAEGER[1], M. JOHN[1], W. J. KORZAN[3], R. P. WATERS[4], T. R. SUMMERS[2];
[1]Biol., Univ. of South Dakota, Vermillion, SD; [2]Biol., Univ. of South Dakota, VERMILLION, SD; [3]Norwegian Univ. of Life Sci., As, Norway; [4]Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA

Abstract: The onset of affective disorders (like anxiety & depression) often coincides with stressful situations; however, individuals react differently to these encounters, making them difficult to diagnose, treat, and assess. Additionally, females are disproportionately affected by these disorders. While social stress is potent and prevalent in both humans and preclinical models used for the study of psychiatric disorders, female mice are difficult to study in the laboratory due to ineffective or exhaustive strategies for the induction of social stress. Using the Stress Alternatives Model (SAM), a behavioral paradigm in which test mice experience social stress from a larger aggressor mice promoting them to diverge into distinct stress-related phenotypes (those that exhibit enhanced fear conditioning/susceptible & those that display active avoidance/resilience), we tested a new technique for inducing social stress in females. Large male retired breeders (CD1 mice) were conditioned to act aggressively toward female mice (C57BL/6) by pairing interactions with a mild shock. The greatest reaction from CD1 aggressors appeared to be observed when the shock was elicited during anogenital sniffing. Despite CD1-induced aggression levels being similar during trials, distinct and important differences were observed between male and female SAM behavior. For example, female mice tend to show more active avoidance behavior and faster active avoidance time. Further, female mice exhibit more motivation toward active avoidance and lower, overall, fear freezing. The stage of the estrous cycle also seemed to be important as female mice in proestrus exhibited lower fear freezing and more social preference in the Social Interaction/Preference (SIP) Test. As we have recently demonstrated that the orexin system is important for mediating stress responses in males, we further assessed this in females. The orexin system is composed of two neuropeptides (Orx_A & Orx_B), produced in the lateral/dorsomedial-perifornical hypothalamus, that act as neuromodulators by targeting two orexin receptor subtypes (Orx_1 & Orx_2). While Orx_A shows equal binding preference for both receptors, Orx_B exhibits a greater affinity for Orx_2. In both

males and females, whole brain (intracerebroventricular) activation of Orx_2 receptors promotes enhanced social preference and resilience in the SIP Test. Together, these results characterize a unique method for promoting social stress in females and suggest a viable option for the treatment of stress-related disorders in both males and females by selectively activating Orx_2 receptors.

Disclosures: C.H. Summers: None. **J.D.W. Yaeger:** None. **M. John:** None. **W.J. Korzan:** None. **R.P. Waters:** None. **T.R. Summers:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.06

Topic: F.04. Stress and the Brain

Support: R01 MH113892

Title: Characterizing the Role of Estradiol in Neural Systems Underlying Social Stress Susceptibility in Female Rats

Authors: *S. E. MOTT[1], B. S. POPE[2], S. K. WOOD[1];
[1]Univ. of South Carolina Sch. of Med., Columbia, SC; [2]Univ. of South Carolina, Columbia, SC

Abstract: Repeated exposure to social stress is a common risk factor for the development of anxiety disorders. Women are twice as likely as men to suffer from anxiety. However, this increased susceptibility in females is confined between the onset of puberty and the end of menopause, suggesting that ovarian hormones may contribute to this disproportionate risk of stress-related disorders. We have shown that witness stress (WS), a model of social stress in which a female rat is subjected to witnessing an aggressive social defeat encounter between a male intruder and a novel male resident, produces anxiety-like behaviors selectively in intact, cycling female rats. Ovariectomized (OVX) females are largely resistant to WS, but estradiol replacement reinstates these anxiety-like behaviors. Moreover, intact females, but not OVX, exposed to WS exhibit a distinct increase in corticotropin releasing factor (CRF) expression in the central amygdala (CeA), a stress sensitive brain region. The current study seeks to understand specific 17-β estradiol (17-βE)-induced neural regulation that may contribute to the heightened susceptibility to social stress among females. Following recovery from OVX or sham surgery, female rats were treated with either 17-βE(10μg/rat, s.c.) or vehicle (0μg/rat, s.c.) and one hour later, were exposed to WS or control handling. WS/control episodes were video recorded to measure burying, freezing, and rearing behaviors, and blood samples were collected one hour after WS/control to assess corticosterone (CORT) levels. Rats were perfused and brains were collected two hours after WS/control. To quantify activity of CRF neurons, brains were sliced

coronally and immunohistochemistry was used to double label for CRF and cFos in various stress sensitive regions. Behavioral responses were shown to be regulated by 17-βE; WS-evoked burying evident in intact females was decreased by OVX and reinstated by 17-βE. However, there was no effect of WS or 17-βE on freezing or rearing behaviors. Additionally, 17-βE increased WS-induced plasma CORT levels. cFos positive CRF expression in the CeA was robustly enhanced by 17-βE during WS, but not control. Conversely, in the CA1 and CA3 subfields of the hippocampus, there was no effect of WS or 17-βE on cFos expression in CRF positive neurons. Analysis of stress sensitive brain regions including the hypothalamic paraventricular nucleus and locus coeruleus are ongoing. Taken together, these findings suggest that 17-βE is paramount in facilitating the anxiety-like responses to WS and support the role of the CeA as a region-specific regulator of these responses.

Disclosures: S.E. Mott: None. **B.S. Pope:** None. **S.K. Wood:** None.

Poster

P612. Stress, Fear, and Anxiety: Pharmacology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P612.07

Topic: G.04. Emotion

Support: VIEP-BUAP grant to CA en Neuroendocrinología BUAP-CA-288

Title: High-yawning rats are more susceptible to dopaminergic D3 agonists than low-yawning males

Authors: *J. EGUIBAR[1], C. CORTES[2], A. TRUJILLO[3], A. UGARTE[4], O. ISIDRO[5]; [1]Benemerita Univ. Autonoma De Puebla, Puebla, Pue., Mexico; [2]Benemérita Univ. Autonoma de Puebla, Puebla, Mexico; [3]Facultad de Ciencias Biológicas, Benemérita Univ. Autonoma de Puebla, Puebla, Pue., Mexico; [4]Inst. de Fisiología, Benemérita Univ. Autonoma de Puebla, Puebla, Pue,, Mexico; [5]Inst. de Fisiología, Benemérita Univ. Autonoma de Puebla, Puebla, Pue., Mexico

Abstract: We selectively inbred two sublines from Sprague-Dawley strain, the high-yawning (HY) had 20 yawns/h and the low-yawning (LY) one order of magnitude lower frequency with just 2 yawns/h. We showed previously that (-)-quinpirole induced more yawns in the HY with respect to LY male rats. The aim of this study was to analyze the effects of two D_3 specific agonists 7-OH-DPAT and PD-128907 on yawning frequency.All the subjects were maintained under standard conditions. Control rats received saline (NaCl 0.9%) or different doses of two D3 agonists by s.c. injection in the dorsal neck region. The experiment last 1 h and observed in a Plexiglass cage and we measured simultaneously yawning and recording them using The Observer XT (Noldus Technologies, The Netherlands) by two trained observers.Our results

showed that both agonists induced higher frequencies of yawning in the HY with respect to LY rats. The 7-OH-DPAT induced the highest yawning frequency with de 5 μg dose and with PD-128907 the 100 μg was the most effective dose. In conclusion, the HY male rats are more sensible to the effects of the specific D3 dopaminergic agonists probably due to differences in the neural circuits involved in the regulation of yawning.

Disclosures: J. Eguibar: None. **C. Cortes:** None. **A. Trujillo:** None. **A. Ugarte:** None. **O. Isidro:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01-DA051650
NIH T32-DA007288
NIH F32-DA053830

Title: Emerging activity dynamics and noradrenergic modulation of prelimbic cortical neuronal ensembles during heroin seeking

Authors: *E. M. DONCHECK, R. I. GRANT, E. V. ROMANOVA, C. W. BOWEN, E. J. SANDAGO, K. T. WINSTON, P. N. SIEGLER, K. M. VOLLMER, L. M. GREEN, M. S. BELL, J. M. OTIS;
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Abstract: Determining the neurobiological basis for relapse in opioid use disorder will facilitate identification of more effective treatment targets. Drug-associated cues are a common relapse trigger, as they can provoke cravings and compulsive drug seeking. Cue-induced drug seeking requires activation of the prelimbic medial prefrontal cortex (PL), which coordinates appetitive behaviors but is dysregulated in substance use disorder. PL is comprised of highly heterogeneous cell types, and this diversity has obscured the precise identification of how PL circuit dynamics orchestrate drug seeking. To address this issue, we developed a head-fixed heroin self-administration procedure to allow longitudinal tracking of PL neuronal activity simultaneous with behavior. To measure the activity of PL projection neurons during this task, we virally labeled these neurons for calcium imaging (AAVdj-CaMKIIa-GCaMP6s) and implanted a GRIN lens dorsal to PL. Subsequent two-photon recordings simultaneous with heroin self-administration reveal reductions in calcium event frequency and amplitude in PL neurons following heroin self-administration, effects which persist through extinction. Interestingly, this hypoactivity was rescued to baseline conditions during cue-induced reinstatement in a manner

that was time-locked to drug-associated cue presentation. As local noradrenergic signaling can rapidly increase PL activity and mediates drug-cue memory retrieval, we hypothesized that inputs from the locus coeruleus (LC) may contribute to the rescue of PL activity during reinstatement. In support of this idea, we find that chemogenetic inhibition of LC-PL axon terminals blocks cue-induced reinstatement of heroin seeking. Surprisingly, this effect was persistent as mice failed to reinstate upon a subsequent cue test, whereas control mice reinstated across multiple tests. Effects of chemogenetic LC inhibition on PL neuronal activity during cue-induced reinstatement revealed differences in excitatory activity dynamics during the CNO test which, like suppression of reinstatement, persisted upon retest. Analyses are underway to track changes in activity dynamics of discrete ensembles and single cells throughout heroin self-administration, extinction, and reinstatement. These findings support the hypothesis that activity in LC-PL noradrenergic axons rescues activity in downstream PL projection neurons for cue-induced reinstatement of heroin seeking.

Disclosures: E.M. Doncheck: None. **R.I. Grant:** None. **E.V. Romanova:** None. **C.W. Bowen:** None. **E.J. Sandago:** None. **K.T. Winston:** None. **P.N. Siegler:** None. **K.M. Vollmer:** None. **L.M. Green:** None. **M.S. Bell:** None. **J.M. Otis:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA T32 Grant DA0728829
NIH/NIDA F31 Grant DA052186
NIDA R01 Grant DA051650

Title: Activity in paraventricular thalamostriatal neurons is necessary and sufficient for the suppression of reward seeking but is dysfunctional following opioid use

Authors: *K. M. VOLLMER[1], L. M. GREEN[1], R. I. GRANT[1], E. M. DONCHECK[1], K. T. WINSTON[1], E. V. ROMANOVA[1], P. N. SIEGLER[1], C. W. BOWEN[1], J. M. OTIS[1,2]; [1]Neurosci., [2]Hollings Cancer Ctr., Med. Univ. of South Carolina, Charleston, SC

Abstract: Suppression of reward-seeking behaviors under inappropriate circumstances is necessary for survival. Opioids have been shown to dysregulate this behavioral inhibition, such that individuals with opioid use disorder (OUD) will compulsively seek opioids even when that behavior is maladaptive or counterproductive. Despite this knowledge, the neural circuits that are responsible for the suppression of reward seeking have not been fully characterized. Recent work from our laboratory, and the work of others, suggests that the posterior paraventricular nucleus of

the thalamus (pPVT) may be involved. Specifically, the pPVT has strong connectivity with the nucleus accumbens (NAc), and pPVT-NAc neuronal activity is often suppressed during reward seeking and feeding but activated when reward seeking would be inappropriate, such as during conditions of stress or satiety. However, it is unclear whether pPVT-NAc activity is necessary and sufficient for the suppression reward seeking, and whether chronic opioid use modifies this circuit's role in motivated behaviors is unknown. Using a sucrose self-administration task, we find that optogenetic stimulation of pPVT-NAc neurons suppresses sucrose self-administration and cue-induced reinstatement of sucrose seeking. Next, we find that optogenetic inhibition of pPVT-NAc neurons unleashes these behaviors under inappropriate circumstances, specifically under conditions of stress or after extinction training. Surprisingly, the inhibition of sucrose-seeking behaviors through pPVT-NAc stimulation was abolished by a single systemic injection of heroin, suggesting that opioids may reorganize or dysregulate this circuit in some manner. In further support of this idea, using an i.v. heroin self-administration task we find that optogenetic stimulation of pPVT-NAc neurons does not suppress cue-, drug-, or stress-induced reinstatement of heroin seeking, unlike sucrose seeking. Finally, using *in vivo* two-photon calcium imaging, we investigated the influence of sucrose versus heroin self-administration on the activity of pPVT-NAc neurons. Overall, we find that pPVT-NAc neuronal activity is highly suppressed in mice that have self-administered heroin, but not sucrose, an effect that persists for weeks beyond their last heroin self-administration session. Overall, our data show that activity in pPVT-NAc neurons is necessary and sufficient for the suppression of natural reward (sucrose) seeking, whereas activity in this circuit is both suppressed by heroin use and incapable of preventing the heroin-seeking behaviors.

Disclosures: K.M. Vollmer: None. **L.M. Green:** None. **R.I. Grant:** None. **E.M. Doncheck:** None. **K.T. Winston:** None. **E.V. Romanova:** None. **P.N. Siegler:** None. **C.W. Bowen:** None. **J.M. Otis:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01-DA051650

Title: Neuronal activity dynamics in prelimbic prefrontal cortex during cue-, drug-, and stress-induced reinstatement of heroin seeking

Authors: *E. V. ROMANOVA, E. M. DONCHECK, R. I. GRANT, E. J. SANDAGO, K. M. VOLLMER, C. W. BOWEN, K. T. WINSTON, P. N. SIEGLER, L. M. GREEN, M. S. BELL, J.

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Abstract: In substance use disorder (SUD), cravings and relapse to drug use emerges in a manner that coincides with dysregulated activity in the prefrontal cortex (PFC). Research using the drug self-administration procedure in rodents confirms that activity within a subregion of PFC, the prelimbic medial PFC (PL-PFC), is necessary for reinstatement of drug seeking provoked by distinct relapse triggers (including drug-associated cues, the drug itself, and stressors). However, the PL-PFC is a highly heterogeneous structure with diverse neuronal ensembles that differentially contribute to behavior, and how these unique neuronal ensemble dynamics might contribute to reinstatement is unclear. Furthermore, despite the convergent requirement of PL-PFC activity for reinstatement, it is unknown whether different relapse triggers provoke drug seeking through overlapping or distinct neuronal ensembles. Here, we paired a novel head-fixed drug self-administration task with simultaneous two-photon imaging of PL-PFC projection neurons to characterize the neuronal ensemble dynamics that differentially evolve during cue-, drug-, and stress-induced reinstatement. Mice received microinfusions of a genetically encoded calcium indicator (AAVdj-CaMKIIa-GCaMP6s) and GRIN lens implantation dorsal to PL to allow visualization of PL-PFC projection neurons across each of the reinstatement tests. Preliminary spectral clustering analyses reveal heterogeneous neuronal ensembles that differentially encode active lever pressing behavior during each of the reinstatement tests, with both excitatory and inhibitory responses for each test. Longitudinal tracking analyses are currently underway to compare PrL-PFC ensemble and single cell activity dynamics across cue-, drug-, and stress-driven reinstatement to determine the extent of their potential overlap and contribution to reinstatement.

Disclosures: E.V. Romanova: None. **E.M. Doncheck:** None. **R.I. Grant:** None. **E.J. Sandago:** None. **K.M. Vollmer:** None. **C.W. Bowen:** None. **K.T. Winston:** None. **P.N. Siegler:** None. **L.M. Green:** None. **M.S. Bell:** None. **J.M. Otis:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.04

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse (NIDA) grants R01-DA051650 to Otis

Title: Characteristics and function of paraventricular thalamus synaptic inputs to nucleus accumbens cell populations in the context of sucrose and opioid seeking

Authors: *L. GREEN, K. M. VOLLMER, R. I. GRANT, E. M. DONCHECK, K. T. WINSTON, E. V. ROMANOVA, P. N. SIEGLER, C. W. BOWEN, E. J. SANDAGO, M. S. BELL, J. M. OTIS;
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Abstract: Suppression of inappropriate or maladaptive reward-seeking behaviors is critical for survival, and yet compulsive opioid seeking and taking occurs despite negative consequences in opioid use disorder (OUD). Recent work from our laboratory, and the work of others, suggests that the posterior paraventricular thalamus (pPVT) plays a crucial role in suppressing inappropriate or maladaptive reward seeking. However, the downstream mechanisms whereby this occurs, and the influence of opioid use on this circuit, remains unclear. Here we use a combination of viral strategies, transgenic mouse lines, patch-clamp electrophysiology, and optogenetics to characterize the innervation of the pPVT projection neurons onto cell-type specific NAc neurons. Overall, data confirms previous work showing that pPVT innervates dopamine D1 receptor (D1+) expressing medium spiny neurons (MSNs), dopamine D2 receptor (D2+) expressing MSNs, and parvalbumin (PV+) expressing interneurons in the NAc. Interestingly, the pPVT-NAc synaptic input onto PV+ interneurons was stronger than that onto MSNs, and was selectively dependent on calcium permeable AMPA receptors (CP-AMPArs). Thus, we next determined if the inhibition of reward seeking caused by pPVT-NAc stimulation requires CP-AMPArs. Data confirms that optogenetic stimulation of pPVT-NAc neurons abolishes sucrose self-administration, whereas self-administration is rescued through intra-NAc infusion of a CP-AMPAr antagonist (IEM-1460), but not antagonists that inhibit D1 signaling (SCH23390) or D2 signaling (Raclopride). Overall, our data support the idea that pPVT-NAc neuronal activity suppresses natural reward (sucrose) seeking through the activation of CP-AMPArs on downstream PV+ interneurons. Ongoing work investigates the influence of opioid use on pPVT inputs to each of the identified downstream cell populations. Together, these data aid in the identification of precise circuits that are required for the suppression of reward seeking and how they are modified in OUD.

Disclosures: L. Green: None. **K.M. Vollmer:** None. **R.I. Grant:** None. **E.M. Doncheck:** None. **K.T. Winston:** None. **E.V. Romanova:** None. **P.N. Siegler:** None. **C.W. Bowen:** None. **E.J. Sandago:** None. **M.S. Bell:** None. **J.M. Otis:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01-DA051650

NIH R25-GM072643

Title: Characterization and functional interrogation of dorsomedial prefrontal neuronal ensembles during conditioned reward seeking

Authors: *R. I. GRANT, E. M. DONCHECK, K. M. VOLLMER, K. T. WINSTON, E. V. ROMANOVA, P. N. SIEGLER, H. HOLMAN, C. W. BOWEN, J. M. OTIS;
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Abstract: Exclusive cell populations within the dorsomedial prefrontal cortex (dmPFC) have been shown to exert control over distinct aspects of reward seeking via divergent activity patterns. However, response heterogeneity exists within these populations that cannot be fully explained by their projection target or genetic profile. To fully characterize this heterogeneity, we used two-photon calcium imaging in awake, behaving mice to measure the activity of dmPFC excitatory output neurons throughout Pavlovian sucrose conditioning. Unsupervised spectral clustering of dmPFC neuronal responses revealed five neuronal ensembles that each encode specific information related to delivery of a sucrose reward, presentation of reward-predictive cues, and behavioral responses to those cues. These ensembles uniquely molded their activity patterns across daily training sessions and stabilized after learning, such that each ensemble's activity could be used to reliably predict unique aspects of the behavior task. These results demonstrate that dmPFC neuronal ensemble dynamics evolve over the course of cue-conditioned reward learning, yet the causal function of each ensemble for reward-seeking behavior remains less clear. To this end, we have begun single-cell optogenetic experiments to functionally manipulate the activity of each dmPFC neuronal ensemble *in vivo*, in the context of both natural reward seeking and drug reward seeking.

Disclosures: R.I. Grant: None. **E.M. Doncheck:** None. **K.M. Vollmer:** None. **K.T. Winston:** None. **E.V. Romanova:** None. **P.N. Siegler:** None. **H. Holman:** None. **C.W. Bowen:** None. **J.M. Otis:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.06

Topic: G.09. Drugs of Abuse and Addiction

Title: The Effects of Kratom Alkaloids on Mesolimbic Dopamine Release

Authors: P. MANUS[1], L. C. RINGER[1], S. A. TOWERS[1], N. B. PAIGE[1], C. R. MCCURDY[2], ***D. B. LESTER**[1];

[1]Psychology, Univ. of Memphis, Memphis, TN; [2]Medicinal Chem., Univ. of Florida, Gainsville, FL

Abstract: Kratom, derived from the plant *Mitragyna speciosa*, is reported to produce stimulant-like effects at low doses and opiate-like effects at high doses. Mitragynine (MG) and 7-hydroxymitragynine (7-HMG) are the major psychoactive constituents of kratom, but the neural mechanisms of these alkaloids are not clear. Given that the effects of kratom are often compared to those of drugs with a high abuse liability, the current study aimed to determine the effect of MG and 7-HMG on reward-related neurotransmission. *In vivo* fixed potential amperometry was used to quantify stimulation-evoked phasic dopamine release in the nucleus accumbens (NAc) of anesthetized male and female mice. During dopamine recordings, mice received an i.p. injection of either MG (1, 15, or 30 mg/kg i.p.), 7-HMG (0.5, 1, or 2 mg/kg i.p.), or vehicle, and dopamine recordings continued for 90 min. Using specific stimulation parameters, dopamine autoreceptor functioning was measured 30 min post injection. Sex effects were observed following MG but not 7-HMG. In males, there was a significant time x MG dose interaction with the low dose seeming to increase dopamine release relative to vehicle and the higher doses towards the end of the recording period. However, follow-up tests revealed no significant dopamine release differences between doses at specific time points. Low dose MG also increased dopamine autoreceptor functioning (+50% from vehicle) in males. Regarding 7-HMG, both sexes responded similarly with a significant time x 7-HMG dose interaction effect on dopamine release. Follow-up tests revealed significant differences between 7-HMG doses from 60-90 min post injection, with the low dose increasing dopamine release (+18% from vehicle) compared to the high dose (-22% from vehicle). 7-HMG did not alter dopamine autoreceptor functioning. Neither MG nor 7-HMG altered the clearance rate of stimulation-evoked dopamine, indicating no drug effect on dopamine transporter functioning. In conclusion, dose effects were observed following both MG and 7-HMG. The low dose of MG (1 mg/kg) affected dopamine release in males more than females, potentially through actions on dopamine autoreceptors, and in both sexes the low dose of 7-HMG (0.5 mg/kg) increased dopamine release while the high dose (2 mg/kg) had the opposite effect. Although these increases in dopamine release are considerably less than those normally observed in stimulants with a high abuse liability, these findings do suggest that MG and 7-HMG alter dopamine transmission. An increased understanding of the neural mechanisms of kratom may provide insight on its potential uses and abuse liability.

Disclosures: P. Manus: None. **L.C. Ringer:** None. **S.A. Towers:** None. **N.B. Paige:** None. **C.R. McCurdy:** None. **D.B. Lester:** None.

Poster

P763. Opioids: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P763.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA08259
NIH MH041256
Hope for Depression Research grant
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Sex and chronic stress alter the distribution of glutamate receptors within rat hippocampal CA3 pyramidal cells following oxycodone conditioned place preference

Authors: *A. DOLGETTA[1], M. JOHNSON[1], K. FRUITMAN[1], L. SIEGEL[1], Y. ZHOU[2], B. S. MCEWEN[3], M. KREEK[2], T. A. MILNER[1];
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Abstract: Ionotropic glutamate receptors, GluN1 and GluA1, have a key role in the neurobiology of opioid addiction and pain modulation. This project elucidates how sex and chronic immobilization stress (CIS) impact the redistribution of glutamate receptors within rat hippocampal CA3 pyramidal cells following oxycodone (Oxy) conditioned place preference (CPP). Four groups of female and male Sprague-Dawley rats subjected to CPP were used: Saline- (Sal) and Oxy-injected (3mg/kg, I.P.) naïve rats; and Sal- and Oxy-injected CIS rats. Sections from each group were labeled with antibodies to GluN1 (BD Biosciences) or GluA1 (Millipore Sigma) and their subcellular distribution within CA3 pyramidal cell dendrites was examined by electron microscopy. Scientific rigor was ensured by having the same experimenters handle rats, labeling tissues from each group in one experiment, and conducting blinded analyses. GluN1: Sal-females compared to Sal-males had elevated cytoplasmic and total dendritic GluN1. Following Oxy CPP, females had reductions in near plasmalemmal, cytoplasmic and total dendritic GluN1, whereas males had no changes. CIS Sal-females and males compared to naïve counterparts had elevated total dendritic GluN1. CIS Oxy-males (which did not acquire CPP) had increased GluN1 on and near the plasmalemma, and in the cytoplasm of dendrites and in dendritic spines. GluA1: There were no differences in GluA1 dendritic distributions in naïve Sal- and Oxy-female and male rats. CIS Sal-males compared to naïve Sal-males had increased plasmalemmal and total dendritic GluA1. Also, CIS Sal-males had more GluA1-labeled spines than CIS Sal-females. CIS Oxy-males compared to CIS Sal-males had an increase in cytoplasmic and total dendritic GluA1, whereas females had no changes. In summary, Oxy CPP reduced the plasmalemmal dendritic GluN1 pools available for ligand binding in females but not males. CIS alone resulted in internalization of GluN1 from the surface of dendrites in females and increased pools of GluA1 available for binding in males. When CIS preceded behavior, available pools of GluN1 increased in Oxy-males. In conclusion, these results show that Oxy CPP alone and stress alone decrease glutamatergic signaling in females without significantly impacting males, and the combination of Oxy CPP and stress enhance glutamatergic signaling in males without significantly affecting females. Combined with our prior studies, this data supports the idea that hippocampal mechanisms affecting opioid addictive behaviors in response to chronic stress differ between males and females.

Disclosures: A. Dolgetta: None. **M. Johnson:** None. **K. Fruitman:** None. **L. Siegel:** None. **Y. Zhou:** None. **B.S. McEwen:** None. **M. Kreek:** None. **T.A. Milner:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.01

Topic: H.01. Attention

Title: Effects of a combination treatment comprising the selective norepinephrine reuptake inhibitor, atomoxetine, and the NMDA receptor co-agonist, D-serine, on sustained attention in male rats

Authors: *Z. V. REDDING, K. SABOL;
Univ. of Mississippi, University, MS

Abstract: Many people live with impaired sustained attention, including individuals with ADHD or schizophrenia. Common treatments for impaired attention include stimulant medications; however, such drugs are associated with negative side-effects and are unsuitable for certain populations (e.g., those with schizophrenia). Stimulants improve attention via effects on dopamine and norepinephrine (NE) systems. Simultaneous targeting of other neurochemical systems could prove a safe and effective strategy. For example, glutamate acting at NMDA receptors is thought to function with NE to promote cognitive processes including perception (Mather et al., 2016). We tested a combination treatment comprising the NE reuptake inhibitor, atomoxetine (ATX), and the NMDA receptor co-agonist, D-serine. Male Sprague-Dawley rats (n=16) performed a two-choice reaction time task. A rat nosepoked in a central aperture for 0.3 - 6.0 sec. A stimulus light then illuminated above a left or right aperture. The rat exited the central aperture and quickly nosepoked beneath the illuminated light for a water reward. Each response was broken into an initiation time (IT) and a movement time (MT) to isolate attentional processes. IT is the time from stimulus presentation until the rat initiates a response. The mode of IT distributions represents attentive sensorimotor processing speed, while IT distribution skew (devmode; mean minus mode) is thought to reflect lapses in attention (Leth-Steensen et al., 2000; Sabol et al., 2003). All rats experienced three counterbalanced sets of repeated measures (saline + saline, D-serine + saline, ATX + saline, and D-serine + ATX). Drugs were administered via i.p. injection 30 minutes before testing. IT mode was unaffected by any treatments. IT devmode and trials completed were reduced and MT was slowed by ATX alone and by the combination of D-serine and ATX. Importantly, the combination treatment reduced IT devmode more than ATX alone without slowing MT or reducing the number of trials completed more than ATX alone. The lack of effects on IT mode suggests that the present treatments do not affect attentive sensorimotor processing speed. On the other hand, simultaneous blockade of NE reuptake and

enhancement of NMDA receptor activation appears to reduce attentional lapses more effectively than either strategy alone. Furthermore, this combination approach does not appear to exacerbate potential movement or motivational side effects of NE reuptake blockade (see Redding et al., 2019). These results could inform the development of safer treatments that improve quality of life in a wide range of people living with impaired attention.

Disclosures: Z.V. Redding: None. **K. Sabol:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.02

Topic: H.01. Attention

Support: R01AG050518

Title: Orexin-1 receptor antagonism alleviates dizocilpine-induced attentional impairments in an NMDA receptor hypofunction model of schizophrenia

Authors: *E. B. MANESS[1], P. C. LITTLE[2], J. A. BURK[2];
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Abstract: Schizophrenia (SZ) is a complex neuropsychiatric condition that is associated with impaired attentional processing and performance. Failure to maintain attention is thought to result from a hyper-reactive basal forebrain cholinergic system that cannot support increasing attentional load, and available antipsychotics often fail to address this pervasive symptom. Acute systemic administration of psychotomimetic NMDA receptor antagonists, which reproduce the NMDA receptor hypofunction observed in SZ, elevates cortical acetylcholine release and disturbs attentional systems. Both orexin (hypocretin) receptor subtypes (orexin-1 and orexin-2) are expressed on corticopetal cholinergic neurons, suggesting that their suppression may lessen basal forebrain excitability and reduce attentional deficits stemming from a hypercholinergic state. A previous experiment in our lab showed that rats trained in a sustained attention signal detection task performed worse and responded less when given the NMDA receptor antagonist dizocilpine (MK-801: 0 and 0.1 ip), and these impairments were reversed following co-administration of the dual orexin receptor antagonist filorexant (MK-6096: 0, 0.1, and 1.0 mM ICV), suggesting orexin receptor antagonism attenuated dizocilpine-induced overstimulation of cortical regions critical for sustained vigilance. To parse the unique role of the orexin-1 receptor, rats in the present experiment (N = 17) received either saline or dizocilpine injections in tandem with the orexin-1 receptor antagonist SB-334867 (0, 3, or 6 µg ICV) prior to attention task performance across six sessions. As was previously found, dizocilpine diminished performance

in signal trials, slowed correct response latency regardless of trial type, and increased the number of omitted trials. Similarly with filorexant, SB-334867 ameliorated dizocilpine-induced deficits in signal trials, and it also quickened response times, though this was more prevalent in males. Unlike filorexant, SB-334867 did not lessen omissions following dizocilpine exposure, perhaps a reflection of the role of the orexin-1 receptor in satiety-linked motivation. Thus, we propose that the orexin-1 receptor is an important target for minimizing the attentional deficits associated with NMDA receptor antagonism.

Disclosures: E.B. Maness: None. **P.C. Little:** None. **J.A. Burk:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.03

Topic: H.01. Attention

Title: Muscarinic M1, but not M4, receptor antagonism impairs divided attention in male rats

Authors: *P. J. MCLAUGHLIN, P. R. NICKLAS, M. L. KIEFER, M. A. WHALEN, M. T. STEWART, E. M. BENNETT, J. R. BECHTOLD, B. A. MILLER;
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Abstract: It has never been more important to understand divided attention, given its role in the multitasking activities that are pervasive in modern living. We first validated a novel operant divided attention task with the muscarinic antagonist, scopolamine. As expected, scopolamine impaired performance of an auditory high response rate sustained attention task, both alone and in the presence of a distracting visual nosepoke task. This is most likely due to greater task difficulty in the latter divided attention version. Our animal model therefore has features of many human divided attention tasks: a main cognitive task that must be continuously monitored, and a secondary distractor activity of a different modality and response set. We then compared antagonists of the M1 and M4 muscarinic acetylcholine receptors, because scopolamine is nonspecific, and because these two receptor subtypes are the targets of putative dementia medications. The M1-preferring antagonist telenzepine impaired divided attention at the lowest dose tested (1.0 mg/kg i.p.), but did not significantly affect performance in the absence of the distractor task. In contrast, the M4-preferring antagonist tropicamide was ineffective (at up to 10.0 mg/kg). Our results strongly implicate the M1 muscarinic receptor in divided attention, and we suggest that M1 agonists may reverse deficits in this important cognitive domain.

Disclosures: P.J. McLaughlin: None. **P.R. Nicklas:** None. **M.L. Kiefer:** None. **M.A. Whalen:** None. **M.T. Stewart:** None. **E.M. Bennett:** None. **J.R. Bechtold:** None. **B.A. Miller:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.04

Topic: H.01. Attention

Support: NIH Grant R01-AG050518

Title: Effects of M1/M4 receptor agonism and M2 receptor antagonism on attention in rats

Authors: G. SMITH, F. REID, ***J. BURK**;
William & Mary, Williamsburg, VA

Abstract: The cholinergic system plays an important role in regulating attentional processing. Diseases such as Alzheimer's Disease are known to degrade cholinergic neurons and result in a wide range of cognitive deficits, including attentional deficits. The present experiments assessed the roles of a M1/M4 receptor preferring agonist, xanomeline, and a M2 receptor antagonist, methoctramine, on attentional performance in rats. Sprague-Dawley adult rats (n = 12) were trained in a two-lever visual sustained attention task in which correct performance required discrimination of a short visual signal compared with trials when no visual signal was provided. After reaching stable task performance, rats were given xanomeline (6.0 mg/kg, ip), methoctramine (1.0 mg/kg, ip), or vehicle prior to task performance. The results indicate that methoctramine, but not xanomeline, has beneficial effects on attentional performance. Overall, these findings suggest that the M2 receptor is a viable target for treating cognitive deficits associated with decreased cholinergic activity. The lack of beneficial effects of xanomeline is consistent with recent evidence that xanomeline administration in vivo produces greater activation of M4 receptors compared with M1 receptors. Future studies investigating the role of M1 in attention should focus on drugs that are more selective for the M1 receptor.

Disclosures: G. Smith: None. **F. Reid:** None. **J. Burk:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.05

Topic: H.01. Attention

Support: NARSAD Young Investigator Grant 28812
NIH grant R01EY005911

Title: Optogenetic activation of norepinephrine neurons in the locus coeruleus enhances visual spatial attention performance in non-human primates

Authors: *S. GHOSH, J. H. R. MAUNSELL;
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Abstract: The locus coeruleus (LC) norepinephrine (NE) neuromodulatory system is implicated in arousal and attention, and regulates activity in many brain areas including visual attentional pathways. Behaviorally relevant sensory stimuli drive phasic spiking of LC-NE neurons, as do motor responses in goal directed behaviors. It is unknown whether the LC-NE neuromodulation of sensory processing plays a causal role in altering perceptual detectability and spatial attention performance. To test this, we selectively expressed excitatory opsins (ChR2) in LC-NE neurons of two rhesus monkeys. We optogenetically activated LC-NE neurons for 400 ms, starting 200 ms before a 200 ms visual stimulus presentation on a random half of the trials while the monkeys performed an attention demanding visual orientation change detection task. In alternating blocks of trials spatial attention was directed to one of two locations in opposite visual hemifields. In separate experimental sessions, we recorded from population of multiple LC-NE neurons and measured stimulus-locked sensory responses while the monkeys did the same attention task. We found that stimulus-locked optogenetic activation of LC-NE neurons enhances visual spatial attentional performance. Perceptual detection of stimulus orientation-changes in the visual hemifield contralateral to the stimulated LC was enhanced when the animal's attention was directed to that hemifield (mean ± SEM change in behavioral sensitivity (d'): monkey S, 0.64 ± 0.05, N = 10, $p < 10^{-6}$; monkey P, 0.37 ± 0.05, N = 11, $p < 10^{-4}$). In contrast, LC activation had no detectable effect on the animal's discrimination of ipsilateral orientation changes. This unilateral effect was also seen when monkeys directed equal attention to both stimulus locations. Spike responses (n = 53) recorded from LC-NE neurons in these animals showed stronger sensory stimulus-locked firing when monkeys directed their attention to the contralateral visual hemifield location compared to the ipsilateral location. Increased spiking of LC-NE neurons associated with the shift in spatial attention and the unilateral effect of optogenetic activation of LC-NE neurons on attention performance are consistent with the ipsilateral LC connectivity with the forebrain attention control areas. Together, these results demonstrate that the phasic LC activity can rapidly modulate attentional behavior with spatial specificity.

Disclosures: S. Ghosh: None. **J.H.R. Maunsell:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.06

Topic: H.01. Attention

Support: R01NS107355
R01NS112200

Title: A graded yet variable relationship between pupil diameter and locus coeruleus activity

Authors: *J. MCBURNEY-LIN, M. MEGEMONT, H. YANG;
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Abstract: A longstanding hypothesis in the field of arousal and attention involves the relationship between pupil diameter and activity in the locus coeruleus (LC), where pupil diameter is often treated as a non-invasive readout of LC activity (e.g., Aston-Jones and Cohen, 2005; Gilzenrat et al., 2010; Preuschoff et al., 2011; Konishi et al., 2017; Clewett et al., 2018; Aminihajibashi et al., 2020). However, to the best of our knowledge, we do not know whether or how pupil diameter is linked to LC activity in a graded manner, or to what extent this relationship may hold. Furthermore, we do not know the extent to which pupil diameter can be used to make accurate inference of LC activity. To address these questions, we recorded the spiking activity from optogenetically-tagged single units (n = 17) from LC simultaneously with pupil tracking in head-fixed mice (DBH-Cre;Ai32). We established a graded relationship between pupil size changes and LC spiking activity, where pupil dilation increased monotonically with the number of LC spikes. However, substantial variability exists in this relationship such that ideal observer analysis cannot accurately predict the majority (> 90%) of LC spiking from pupil size changes. Next, to test whether pupil size changes better reflect population-level LC activity, we optogenetically activated groups of LC neurons and quantified the evoked pupil responses. We found that pupil responses exhibited large session-to-session fluctuations to identical optical stimulation, in sharp contrast to the stable LC responses. Importantly, baseline pupil diameters were similar and thus cannot explain the differences in evoked pupil responses. To understand what may underlie the variable pupil responses, we examined a subset of pupil-LC recordings where mice were trained to perform a go/no-go tactile detection task (Yang et al., 2016). We found that the variations in motivational state during task performance at least partially explained the variations in the pupil-LC relationship. Together, our data show that substantial state-dependent variability exists in the overall graded relationship between pupil size and LC, which seriously impedes using pupil diameter to make accurate inference of LC activity. Our current study substantiates and extends our previous findings that LC activity and pupil size changes represent distinct task-related events (Yang et al., 2021).

Disclosures: J. McBurney-Lin: None. **M. Megemont:** None. **H. Yang:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.07

Topic: H.01. Attention

Support: CIHR
OGS

Title: Effects of psilocybin and the serotonin 5-HT_{2A} antagonist M100,907 on attention measured in the rodent continuous performance task

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[1]Psychology, [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [3]Biopsychology, Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: In humans, impaired attention is a feature of several neuropsychiatric disorders including substance abuse, major depression and anxiety disorders. Attention has been shown to be modulated by the serotonin neurotransmitter system. Recently, the serotonin 5-HT_{2A} receptor agonist and hallucinogen psilocybin (PSI) has been investigated as a treatment for disorders that are characterized in part by attention deficits. However, few studies have investigated whether psilocybin has a direct effect on attention (Barrett et al., 2020, Higgins et al., 2021). Using an established test of attention in rodents, the continuous performance task (CPT), we evaluated whether PSI influences the attentional performance of mice. Additionally, as PSI's potential therapeutic effects are thought to arise from 5-HT_{2A} stimulation, we also evaluated the effects of the 5-HT_{2A} receptor antagonist M100,907 for comparative purposes. Mice were trained in a touchscreen-based system to distinguish between target and non-target visual stimuli (2s stimulus duration) for milkshake reward. Once mice reached a stable level of performance, they were tested following injections of PSI (0, 0.3, 1 and 3 mg/kg s.c.) or M100,907 (0, 0.1, 0.3, and 1 mg/kg s.c.) They were then tested following injection with PSI (0, 0.3 and 1 mg/kg) and M100907 (0, 0.1, 0.3) mg/kg on a more difficult version of the CPT with a variable stimulus duration (1, 0.75, 0.5, and 0.25s). Performance was evaluated via signal detection theory measures including hit rate, false alarm rate, target discrimination (d') and response bias (C), as well as perseverative responding. On the basic task the highest dose of PSI decreased target discrimination (lower hit rate) and perseverative responding, and induced a more conservative response bias (less likely to respond). There were no effects of PSI at other doses. M100,907 had no effect on target discrimination, but reduced perseverative responding, while inducing a more conservative response bias. On the more difficult task, overall attentional performance was reduced, PSI had no effects, and M100,907 induced similar performance changes as on the basic task. We also examined the effects of PSI and M100907 on locomotor activity. The highest PSI dose and all M100,907 doses significantly reduced locomotor activity. Taken together the results of these experiments suggest that exposure to M100,907 and high doses of PSI may acutely impact performance on the CPT. However, these results may be in part due to drug-induced locomotor deficits. Further work is required to investigate whether PSI may impact attention in more cognitively demanding tasks or following repeated PSI exposure.

Disclosures: A. Rahbarnia: None. **P.J. Fletcher:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.08

Topic: H.01. Attention

Support: PHS grant R01DA045063

Title: Disrupted choline clearance and sustained acetylcholine release in vivo by a common choline transporter coding variant associated with poor attentional control in humans

Authors: *E. DONOVAN[1], C. AVILA[2], V. V. PARIKH[3], M. FENOLLAR FERRER[4], R. D. BLAKELY[5], M. SARTER[6];
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Abstract: Transport of choline via the neuronal high-affinity choline transporter (CHT; SLC5A7) is essential for cholinergic terminals to synthesize and release acetylcholine (ACh). In humans, we previously demonstrated an association between a common CHT coding substitution (rs1013940; Ile89Val) and reduced attentional capacity as well as attenuated frontal cortex activation. Here, we used a CRISPR/Cas9 approach to generate mice expressing the I89V substitution and assessed, using in vivo cortical choline biosensing, CHT-mediated choline transport, and ACh release. CHTmediated clearance of choline in mice expressing one or two Val89 alleles was reduced by over 7-fold relative to wild type (WT) mice, suggesting dominant-negative effects. Choline clearance in CHT Val89 mice was further reduced by neuronal inactivation. Deficits in ACh release, 5 and 10 min after repeated depolarization at a low, behaviorally relevant frequency, support an attenuated reloading capacity of cholinergic neurons in mutant mice. The density of CHTs in total synaptosomal lysates and neuronal plasma-membrane-enriched fractions was not impacted by the Val89 variant, indicating a selective impact on CHT function. Consistent with this hypothesis, structural modeling revealed that Val89 may attenuate choline transport by changing the ability of choline to induce conformational changes of CHT that support normal transport rates. Our findings suggest that diminished, sustained cholinergic signaling capacity in the frontal cortex underlies perturbed attentional performance in individuals expressing CHT Val89. Our work supports the utility of the CHT Val89 mouse model as a valuable model to study heritable risk for cognitive disorders arising from cholinergic dysfunction.

Disclosures: E. Donovan: None. **C. Avila:** None. **V.V. Parikh:** None. **M. Fenollar Ferrer:** None. **R.D. Blakely:** None. **M. Sarter:** None.

Poster

P773. Mechanisms of Attention: Cholinergic System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P773.09

Topic: H.01. Attention

Support: NIH Grant 1F31NS122428-01

Title: Utilizing fluorescent reporters of neuromodulator activity to assess brain-state-related cortical acetylcholine and norepinephrine dynamics

Authors: *E. NEYHART, C. L. SMITH, M. A. GALDAMEZ, N. ZHOU, Z. H. MRIDHA, J. DE GEE, M. J. MCGINLEY, J. REIMER;
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Abstract: The brain naturally transitions between a variety of arousal states throughout the day which affect the way information is processed. Within the state of wakefulness there are subtle, transient substates which fluctuate on the order of minutes to seconds and are accompanied by rapid changes in sensory processing. Cortical activity of neuromodulators such as acetylcholine (ACh) and norepinephrine (NE) have been associated with these transient substates and their behavioral correlates (locomotion and pupil diameter), and are thought to have a direct role in influencing changes in sensory processing. However, it is not yet understood on a fine spatiotemporal scale when and where ACh and NE are spontaneously available across the cortex during these rapidly fluctuating substates. Recently-developed G-protein-coupled-receptor-based fluorescent reporters of neuromodulator activity, such as GACh and GrabNE, offer the potential to resolve these questions. However they have yet to be characterized extensively in vivo. The current study utilized in vivo two-photon imaging to record activity of virally-expressed GACh and GrabNE in various cortical regions of male and female C57/BL6 mice. Both agonists and antagonists of ACh and NE were injected to the visual cortex to obtain in vivo dose-response curves of the sensors. GACh and GrabNE activity were also recorded simultaneously with mutated versions of the sensors, green fluorescent protein alone, and another ACh fluorescent reporter (iAChSnFR) as a control. Then, heterogeneity of spontaneous ACh and NE activity in visual, somatosensory, auditory, and motor cortex was assessed, as well as the temporal relationship of these neuromodulators to locomotion and pupil diameter. Findings regarding the responses of GACh and GrabNE to actual changes in cortical neuromodulator levels will help to interpret the results of further studies utilizing these sensors in vivo. Understanding the spatial scale at which cortical ACh and NE spontaneously fluctuate across waking states sheds light on how different modes of information processing are being produced. Furthermore, better-defining

the link between pupil diameter, locomotion, and actual activity of ACh and NE will help validate these external correlates of brain state changes as reporters of neuromodulator activity.

Disclosures: E. Neyhart: None. **C.L. Smith:** None. **M.A. Galdamez:** None. **N. Zhou:** None. **Z.H. Mridha:** None. **J. De Gee:** None. **M.J. McGinley:** None. **J. Reimer:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.01

Topic: H.08. Learning and Memory

Support: NIH R01-DA-042057
I01-RX-002252-03
1F30MH122093-01A1

Title: Quantification of Fos neuroactivity using photoacoustic imaging in vivo with validation by ex vivo immunofluorescence

Authors: *J. I. MATCHYNSKI[1], R. MANWAR[2], N. SADIK[1], K. M. MAKKI[3], T. J. CILLEY[1], A. R. WOZNICKI[3], S. KALLAKURI[1], B. T. HOPE[4], K. AVANAKI[2], S. A. PERRINE[5], A. C. CONTI[5];
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Abstract: Current functional neuroimaging techniques, such as functional magnetic resonance imaging, rely on indirect consequences (the hemodynamic response) to quantify neural activity. With the development of a photoacoustic (PA) imaging system and use of transgenic rodent technology, we propose a more direct method to map and quantify activated neurons using a Fos-LacZ transgene reporter system in rats. Fusion of Fos with the lacZ gene gives active (Fos+) cells the ability to cleave pro-chromogenic substrates, such as X-gal, into PA-detectable products. In this study, we quantified the neuroactivity-based PA signal in Fos-LacZ transgenic rats following cued fear conditioning or chemical and behavior control conditions following intrathecal administration of X-gal. Immunohistochemistry for Fos, NeuN, and beta-galactosidase was quantified in a subset of animals for validation. Homozygous Fos-LacZ Wistar female and male rats (N = 80 total) underwent acquisition of cued fear conditioning and intrathecal X-gal administration 60 minutes after the conclusion of fear conditioning. Twenty-four hours later, rats were anesthetized and a cranial window was created in the skull. PA imaging of Fos-dependent neuroactivity in the medial prefrontal cortex (mPFC) *in vivo* was immediately conducted and the signal strength was quantified. All PA imaging used an 18.5

MHz, 128 element L22-14v linear array ultrasound transducer to record PA signal produced by pulsed laser illumination directed with a custom optical fiber bundle. A second group of animals, identically prepared (N = 48), was perfused 90 minutes after fear conditioning or control conditions. Brain tissues were triple labeled with antibodies against NeuN, Fos, and beta-galactosidase for e*x vivo* validation using cell count analysis and colocalization. Images were quantified using Matlab and the FIJI version of ImageJ. We report and compare fear response and Fos-expression from quantified *in vivo* PA images after X-gal administration and quantify triple-labeled confirmatory *ex vivo* immunofluorescence images acquired. Our data demonstrate significant differences in fear responding, PA signal strength, and cell counts between fear conditioned animals and vehicle or behavioral controls. Correlational data of fear response and Fos-expression *in vivo* and *ex vivo* is also reported. Using this novel functional molecular photoacoustic approach, we demonstrate an *ex vivo* validated method of monitoring activated (Fos+) neurons, i.e. neuronal ensembles, *in vivo* with high spatial resolution and specificity.

Disclosures: J.I. Matchynski: None. **R. Manwar:** None. **N. Sadik:** None. **K.M. Makki:** None. **T.J. Cilley:** None. **A.R. Woznicki:** None. **S. Kallakuri:** None. **B.T. Hope:** None. **K. Avanaki:** None. **S.A. Perrine:** None. **A.C. Conti:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.02

Topic: H.08. Learning and Memory

Support: NIH Grant R01-DA-042057
VA Merit Award I01-RX-002252-03

Title: Repetitive traumatic brain injury induces chronic anxiety, forepaw thermal sensitivity and neuroinflammation in rats

Authors: *S. KALLAKURI[1,3], N. SADIK[1,3], A. C. CONTI[1,3], K. E. BOSSE[1,3], C. BIR[2], S. A. PERRINE[1,3];
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Abstract: Repetitive blast traumatic brain injuries (rbTBI) are a common cause of chronic pain as well as anxiety and cognitive deficits in many U.S. Veterans. Persistent neuroinflammation has been postulated to be a key player; however, much of the current knowledge on long-term rbTBI changes are primarily related to behavioral changes with a limited focus on underlying inflammation. To address this knowledge gap, this study assessed neuroinflammation in conjunction with changes in pain and anxiety at chronic post-rbTBI timepoints. Anesthetized

Sprague Dawley rats were subjected to rbTBI (one blast exposure/day for 3 consecutive days using a shock tube) or sham procedures. Following rbTBI or sham procedures, surface righting duration was immediately measured, and 1 or 3 months after rbTBI or sham procedure, anxiety-like behavior was measured by elevated plus maze (EPM) and pain behavior was tested by forepaw thermal withdrawal latency to radiant heat exposure. Perfused, fixed brain sections were collected following behavioral testing and assessed for neuroinflammation using immunofluorescence for microglial and astrocytic proliferation in the prefrontal cortex. Ionized calcium-binding adapter molecule 1 and glial fibrillary acidic protein were measured in astrocytes and microglia. Acutely, rbTBI animals showed a prolonged duration to surface right compared to sham animals. At both 1- and 3-month post-blast, rbTBI rats displayed anxiety-like behavior, indicated by decreased open arm time/entries, and they had higher pain sensitivity as indicated by shorter thermal withdrawal latency compared to sham-controls. A high number of microglia and astrocytes were observed in rbTBI animals compared to sham. Overall, these data suggest the development of anxiety-like and thermal sensitivity changes long after rbTBI which may be mediated by the underlying chronic inflammatory state. Future studies will be directed at understanding the effects of rbTBI on opioid-taking behaviors and the ensuing neuropathology.

Disclosures: S. Kallakuri: None. **N. Sadik:** None. **A.C. Conti:** None. **K.E. Bosse:** None. **C. Bir:** None. **S.A. Perrine:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.03

Topic: H.08. Learning and Memory

Support: NIH R01-DA-042057 (Perrine)
VA Merit Award I01-RX-002252-03

Title: Differences in cognitive behaviors in a Barnes maze assay between female and male rats

Authors: *C. DAVIDSON[1], A. GHEIDI[1], S. KALLAKURI[2], S. SIMPSON[2], N. SADIK[2], S. A. PERRINE[2];
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Abstract: Drug addiction is a major societal issue that negatively impacts an individual's well-being and relationships. A growing body of literature suggests the existence of differential trajectories in the different phases of addiction between females and males. There is, however, a paucity of knowledge on the impact that addictive substances, such as opioids, have on cognitive function, particularly on spatial learning and memory during drug withdrawal in females and males. In fact, preclinical studies that examine sex differences in cognition are lacking and those

examining learning and memory, particularly using a Barnes maze, are even sparser. As a precursor to studies we are currently undertaking that examine opioid-induced cognitive changes, we assessed baseline cognitive function using a Barnes maze assay in both the sexes. Female and male Sprague-Dawley rats were trained to reach a hidden goal box on a custom-made 20-hole Barnes maze apparatus. Each animal was subjected to 3 trials per day for a total of 5 days. To encourage rats to locate the hidden area, aversive visual (bright light) and auditory (80 dB white noise) cues were used. Preliminary results indicate reduced time to reach the goal box with progressive training in both the sexes over days. Also, male rats showed a prolonged duration to reach the goal box compared to female rats on the second day, otherwise, no other significant differences were observed. Vaginal lavage and cytological characterization of estrous phase have been collected and are being analyzed for insights into associated hormonal cycling interactions with behavior in females. Future studies will be directed at understanding the effect of drugs of abuse, including fentanyl and oxycodone, on cognitive function in females and males. Additionally, we intend to more directly investigate the impact of noradrenergic signaling on Barnes maze behavior following opioid administration using the noradrenergic neurotoxin N--N-ethyl-2-bromobenzylamine (DSP-4). Conceptually this will be examined during drug-withdrawal as it is often considered a stressor that leads to relapse behavior in addictions.

Disclosures: C. Davidson: None. **A. Gheidi:** None. **S. Kallakuri:** None. **S. Simpson:** None. **N. Sadik:** None. **S.A. Perrine:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.04

Topic: H.08. Learning and Memory

Support: NIH R01-DA042057
VA Merit Award I01-RX-002252-03

Title: Neuronal ensemble in the medial prefrontal cortex is involved in anxiety-like behavior in rats with a history of binge cocaine administration and drug-abstinence

Authors: *A. GHEIDI[1], A. T. TROMBLEY[1], N. SADIK[1], S. KALLAKURI[1], J. I. MATCHYNSKI[3], K. KARAVIDHA[1], S. BRUMMELTE[2], B. T. HOPE[4], S. A. PERRINE[1]; [2]Dept. of Psychology, [1]Wayne State Univ., Detroit, MI; [3]TNP, Wayne State Univ. Sch. of Med., Detroit, MI; [4]Behav Neurosci, NIH/NIDA, Baltimore, MD

Abstract: Cocaine abuse remains a public health problem with no FDA-approved medications to treat cocaine use disorder. Previous results show that rats given chronic binge-pattern cocaine administration display a disinhibited hyper-exploratory phenotype in novel environments,

indicative of anxiety-like behavior. A growing body of evidence also suggests that targeted neuronal ensemble ablation in the medial prefrontal cortex (mPFC) can affect cocaine seeking and taking behaviors. Therefore, we assessed the contribution of mPFC neuronal ensembles in the disinhibited hyper-exploratory phenotype observed in a rat model of chronic binge cocaine administration. Female and male heterozygote *Fos-LacZ* Wistar rats were administered either cocaine (15 mg/kg/injection) or saline (1 ml/kg/injection) for 14 days using a binge-pattern administration paradigm (3 times daily at 1-hour intervals) followed by 14 days of forced abstinence (in the home cage). Injections took place in locomotor activity chambers (LMA) on days 0, 1 and 14 and their respective home cages on other days. Rats were then assessed for novelty induced exploration in a black plexiglass open field box. After 90 minutes, the time point of maximum Fos expression and hence β-galactosidase expression in *Fos-LacZ* rats, Daun02 or vehicle was intracranially-injected bilaterally into the mPFC. After 3 days of rest in the home cage, all rats were tested for cocaine-induced hyper-exploratory behavior on a raised elevated plus maze consisting of closed/open arms. Our results show that a sexually dimorphic LMA response to cocaine occurred over the 14 days of cocaine administration, with females showing greater behavioral sensitization. Additionally, rats treated with cocaine displayed higher anxiety-like behavior by spending less time in the center of open field following 14 days of cocaine withdrawal. In the elevated plus maze, cocaine-treated rats who received vehicle injections continued to show anxiety-like behavior, but Daun02 chemoablation of mPFC neuronal ensemble attenuated cocaine-induced anxiety-like behavior by increasing time/entries in open arms. The finding that ensemble ablation of one context carries over to a new context is an interesting finding and suggests a shared neuronal ensemble in anxiety provoking stimuli of the mPFC. Furthermore, neuronal ensembles that underlie anxiety-like behavior during cocaine withdrawal can contribute to dysfunction of the mPFC that plays a role in drug-relapse.

Disclosures: A. Gheidi: None. **A.T. Trombley:** None. **N. Sadik:** None. **S. Kallakuri:** None. **J.I. Matchynski:** None. **K. Karavidha:** None. **S. Brummelte:** None. **B.T. Hope:** None. **S.A. Perrine:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.06

Topic: H.08. Learning and Memory

Support: NIH Grant R56MH119116-01

Title: Neural representation of latent cause in credit assignment

Authors: *Y. L. ZHANG, E. D. BOORMAN, P. P. WITKOWSKI, S. A. PARK, A. C. LEONES;
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Abstract: Humans have a remarkable capacity to use an internal model of the environment to make inferences about unseen outcomes. Such an adaptive behavior may depend on the ability to update our models by learning the causal relationships between the received outcome and the underlying latent cause from choice. For example, upon eating a delicious nectarine, a model-based agent would assign credit to the summer season as the latent cause of ripe stone fruits, leading to the inference that peaches are also in season. How the human brain assigns credit for important outcomes to such latent causes is unknown. We scanned hungry healthy participants (N=25) while they tracked two probabilistic systems of stimulus-reward associations for two desserts, with each system comprising two stimuli of different visual categories but sharing the same probabilities for leading to each reward. We hypothesize that 1. At feedback time, the causal choice reactivates in different subregions within the occipital-temporal cortex depending on the visual category it belongs to (face, body part, scene, tool), as well as an inferred stimulus sharing the same latent cause 2. The underlying latent cause is reinstated in the lateral orbitofrontal cortex (OFC). Behavioral results from a Bayesian learning model and logistic regressions analyses show that participants efficiently learned to track reward probabilities both from their choice and the inferred stimulus in the same system (direct and indirect choices, 1-back: $t(24) > 3.5$ $p<0.001$). An univariate analysis of the Kullback-Leibler divergence as a measure of belief confirmation of stimulus-outcome probabilities at feedback shows significant effects in the OFC, ventromedial prefrontal cortex and hippocampus ($t(24)>4.3$, $p<0.0001$, uncorrected). To test for hypothesis 2, we used multivariate pattern analysis with a cross-validation approach to test for a reinstatement of the causal choice identity at feedback time across runs. Left lateral OFC and hippocampus show a significant decoding accuracy relative to label-shuffled permutations, consistent with a role of OFC in choice reactivation during credit assignment ($t(24)>3.7$, $p<0.001$, uncorrected). Ongoing analyses will test the brain regions responsible for credit assignment to the inferred stimulus at feedback time and a common representation for the latent cause. These findings support a model whereby choices and inferred causes are reinstated at feedback time, coincident with prediction errors, to drive plasticity between co-active neural ensembles for the outcome and cause in the service of learning.

Disclosures: Y.L. Zhang: None. **E.D. Boorman:** None. **P.P. Witkowski:** None. **S.A. Park:** None. **A.C. Leones:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.07

Topic: H.08. Learning and Memory

Support: Wellcome Trust 110157/Z/15/Z

Title: Capturing mediodorsal thalamus and orbitofrontal cortex interactions in primate cognition

Authors: B. A. L. PERRY[1], J. MENDEZ[1], *A. MITCHELL[2];
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Abstract: Anatomical evidence in mammals shows that the mediodorsal thalamus (MD) is a key component within wider prefrontal thalamocortical networks. Accordingly, damage to, or disruption of MD function impairs specific types of complex learning and decision making in primates. Yet, the underlying mechanisms behind the influence of MD on the frontal cortex during these higher order cognitive processes remain unknown. In the current study, two male rhesus macaques were trained to perform a touchscreen-based visuo-spatial discrimination learning task prior to having recording chambers implanted above the MD and orbitofrontal cortex (OFC). All experimental procedures were approved and conducted under a United Kingdom Home Office Project Licence issued to Dr Mitchell. After recovery, we established the precise coordinates to target the MD and the OFC using magnetic resonance imaging (MRI). Once the animals had re-established their pre-procedure learning rates, we recorded task related neural activity from the MD and OFC using multi-contact probes that were lowered before each testing session. MD and OFC units were modulated during specific phases of a trial, responding differently to the trial onset, when the visuo-spatial images first appeared on the touchscreen; when the monkey made a response to the touchscreen; and before or after the delivery of the smoothie reward for correct trials. We also observed some MD and OFC units progressively modulated their responses throughout the session, coinciding with improved cognitive performance. Finally, during correct trials, some MD units were phase-locked to OFC local field potentials, suggesting prefrontal thalamocortical interactions support learning. Our evidence highlights different, but complementary and interactive neural activity between the MD and OFC during learning of a visuo-spatial discrimination task.

Disclosures: B.A.L. Perry: None. **J. Mendez:** None. **A. Mitchell:** None.

Poster

P795. Prefrontal Networks: Ventromedial Prefrontal Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P795.08

Topic: H.08. Learning and Memory

Support: NIH R01 NS091010
NIH R01 DC018545

NIH R03 MH120426
NIH R01 EY025349
NIH P30 EY022589
NSF 1940181
David & Lucile Packard Foundation

Title: Orbitofrontal cortex mediates meta-learning of reinforcement learning

Authors: *R. HATTORI[1,2,3,4], S. CHEN[1,2,3,4], H. YOU[1,2,3,4], M. HATTORI[1,2,3,4], J.-H. CHOI[1], B. LIM[1], T. KOMIYAMA[1,2,3,4];
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Abstract: The meta-reinforcement learning (meta-RL) framework, which uses RL over fast and slow timescales, has been successful in training deep RL networks that generalize to new environments. This framework uses slow plasticity-based RL to build a recurrent network that performs fast trial-by-trial RL in an activity-based, plasticity-free manner. It has been hypothesized that the recurrent neural network in the prefrontal cortex may mediate meta-RL in animals, but the evidence is scarce. Here we show that the orbitofrontal cortex (OFC) of the prefrontal area is critically involved in both fast and slow aspects of meta-RL in mice. We trained mice and artificial meta-RL networks on a probabilistic reversal learning task across sessions during which they showed meta-learning and improved their trial-by-trial RL policy. By optogenetic suppression of synaptic plasticity, we showed that synaptic plasticity in OFC was necessary for this across-session meta-learning of RL but not for the within-session trial-by-trial RL in experts. After the meta-learning, OFC population activity robustly encoded history-based value signals, and transient optogenetic OFC inactivation impaired the ability of mice to exploit the value on each trial. Longitudinal tracking of OFC neural activity during training by in vivo 2-photon calcium imaging through GRIN lens revealed that the across-session meta-learning slowly stabilizes population value coding in accordance with the ongoing behavioral policy. All these experimental results from OFC were consistent with simulations from artificial meta-RL networks. Our results indicate that two distinct RL algorithms with distinct neural mechanisms and timescales coexist in OFC to support adaptive decision making.

Disclosures: R. Hattori: None. **S. Chen:** None. **H. You:** None. **M. Hattori:** None. **J. Choi:** None. **B. Lim:** None. **T. Komiyama:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.01

Topic: H.06. Social Cognition

Support: Science of Learning and Art of Communication (SLAC) Innovation Award

Title: Social learning in a novel operant conditioning paradigm in rats

Authors: *R. TROHA[1], M. GOWDA[1], J. LEVIN[1], A. BUZZANCA[1], Q. HU[1], E. J. MARKUS[2]; [1]Univ. of Connecticut, Storrs, CT; [2]Univ. of Connecticut, Storrs Manfld, CT

Abstract: Social learning is an important survival mechanism. For example, learning to find food in an environment requires less time and energy and encompasses less exposure to danger when following a conspecific familiar with the environment. In humans, social imitation plays an important role in development (Bandura 1961). Although this form of learning has been well-documented in humans, most research in animals has focused on social transmission of fear**.** Previous research in our lab focused on development of a behavioral task in which an observer animal learned to find food on a T-maze by watching a demonstrator animal. Although observers eventually learned the task, much training was needed and effects were minimal. The goal of the current study was to develop an improved behavioral paradigm in which socially driven appetitive conditioning can be studied. Animals were trained in an apparatus consisting of two conjoined operant chambers, with one chamber containing an observer rat and the other containing a demonstrator rat. The observer was trained to respond at a nose poke in their own chamber on the same side which the demonstrator responded. Therefore, the observer learned to watch the demonstrator response and mimic the behavior in its own chamber. An additional non-social control task was used in which animals were trained to observe a plastic ball being illuminated. An important feature of this task is the ability to expose animals to many trials in one training session. This resulted in faster and more robust learning compared to previous work in our lab. Once learning was achieved, behavior was then analyzed using DeepLabCut. This deep learning approach allowed precise tracking of both the observer and demonstrator, even under constantly changing lighting conditions. Overall, observer animals performed better when observing a trained demonstrator compared to the ball stimulus. Results also showed that observers oriented themselves towards the demonstrator during the demonstrator response period. This orientation was gradual, in that observers oriented more towards the demonstrator as the demonstrator approached the nose poke receptacle. Results also showed that observers oriented more towards a demonstrator rat compared to an inanimate cue object. The initial orientation of the observer animal at the start of the trial also significantly affected response accuracy. This novel task may be used in future experiments to probe the neural circuits underlying this form of learning. These future results could have implications for treatment of disorders where social deficits are seen.

Disclosures: R. Troha: None. **M. Gowda:** None. **J. Levin:** None. **A. Buzzanca:** None. **Q. Hu:** None. **E.J. Markus:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.02

Topic: H.06. Social Cognition

Support: Peter and Carmen Lucia Buck Foundation
UCRF
UConn IDEA Grant

Title: Social factors influence solo and dyad male rat pairs during novel open field exploration

Authors: *Q. HU, S. LEE, S. AHMED, L. HORBAL, T. A. PIETRUSZEWSKI, E. J. MARKUS;
Univ. of Connecticut, Storrs, CT

Abstract: Exploration of a new environment is an essential component of animal behavior, providing potentially crucial information regarding sources of food, shelter, and mates. Eilam and colleagues (Weiss et al., 2015) found that male rats explore familiar arenas differently in the presence of another male rat. In the current study, we examined interactions of paired male rats during exploration of a novel environment. Nine-month old male F344 rats (Harlan, IN), with free access to food and water were used for this experiment. Animals were paired for 7 days prior to and throughout testing. The open field exploration was assessed using a Plexiglas enclosure (70.5cm L X 70.5cm W) with a white plastic floor of the same area. During experimental trials, either one or two rats were placed in the southeast corner of the open field and allowed to explore for fifteen minutes. Videos of the trials were then analyzed using DeepLabCut (Mathis et al., 2018) to obtain position information of the subjects. The software allowed for tracking of multiple body parts of multiple animals. Rats were placed in the novel environment either individually or in pairs. This was repeated the following day. On the first (novel) day, solo rats traveled a greater distance, explored a larger area, and spent less time in the outer zone than paired rats. Stranger rat pairs spent more time in the outer zone and interacting with their nose to nose in close proximity than familiar pairs. These effects were not found on the following day.

Disclosures: Q. Hu: None. **S. Lee:** None. **S. Ahmed:** None. **L. Horbal:** None. **T.A. Pietruszewski:** None. **E.J. Markus:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.03

Topic: H.06. Social Cognition

Support: NIH Grant R01-MH104602
NIH Grant R01-MH106629

Title: A direct lateral entorhinal cortex to hippocampal CA2 circuit conveys social information required for social memory

Authors: *J. LOPEZ-ROJAS[1], C. A. DE SOLIS[1], F. LEROY[2], E. R. KANDEL[3], S. A. SIEGELBAUM[4];
[2]Dept. of Neurosci., [3]Jerome L. Greene Sci. Ctr., [1]Columbia Univ., New York, NY; [4]Dept of Neurosci., Columbia Univ. Coll P & S, New York, NY

Abstract: The hippocampus is essential for forming and storing declarative memories, including social memory, the ability of an animal to recognize and remember a conspecific. To serve this mnemonic function, the hippocampus relies on a variety of external inputs. Despite recent advances in our understanding of the brain circuits supporting social memory, little is known about the role of the entorhinal cortex (EC), which provides the primary source of multimodal sensory input from neocortex to hippocampus. Here we report that the classical trisynaptic pathway, in which information from EC is powerfully but indirectly routed to hippocampal CA1 and CA2 pyramidal neurons (PNs) through dentate gyrus (DG) and CA3 (EC-DG-CA3-CA1/CA2), is dispensable for social memory. Rather, social memory depends on the direct relay of social information from EC to dorsal CA2 PNs, which receive uniquely strong direct inputs from EC compared to their CA1 and CA3 neighbors. Moreover, the information routed to CA2 is specifically provided by inputs from the lateral EC (LEC), rather than from medial EC. Thus, our findings highlight the behavioral relevance of the strong direct LEC pathway to CA2 in mediating the integration of complex sensory cues required for social memory.

Disclosures: J. Lopez-Rojas: None. **C.A. de Solis:** None. **F. Leroy:** None. **E.R. Kandel:** None. **S.A. Siegelbaum:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.04

Topic: H.06. Social Cognition

Support: NIMH F30 5F30MH120922-02

Title: The geometry of social and spatial representations in the hippocampal region dorsal CA2

Authors: *L. BOYLE[1], L. POSANI[1], S. IRFAN[2], S. A. SIEGELBAUM[1], S. FUSI[1];
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Abstract: The ability to recognize other individuals is a crucial component of social behavior. The hippocampus plays an important role in this process, as silencing the hippocampal subregion dorsal CA2 impedes the storage, consolidation, and recall of social recognition memory. However, it is not clear what kind of information and which modalities are represented in the CA2 region during social interaction. Specifically, there is an open question whether and how social novelty and conspecific identity are represented in CA2, and how these social variables interact with non-social spatial features in the neural code. To investigate these questions, we used the Amigo2-Cre mouse line and performed miniscope calcium recordings of dorsal CA2 pyramidal neuron activity in awake, behaving mice exposed to different combinations and placements of novel and familiar individuals. We then characterized CA2 neural population representations using a decoding approach to explore the relationship between social and non-social variables during social interaction. We found that the identity of novel as well as familiar conspecifics is consistently encoded in neural activity in dorsal CA2 during social interaction. These representations are abstract with respect to spatial features, as they are maintained across different positions of interacting individuals. By comparing novel and familiar individuals, we found that social novelty is robustly encoded in neural activity, and that this "social novelty signal" is abstract with respect to spatial as well as social identity variables, as the difference in novelty could be consistently decoded across different positioning and different identities of the interacting individuals. Our results suggest that CA2 robustly encodes both social novelty and identity across different contexts and that the geometry of these neural representations is abstract.

Disclosures: L. Boyle: None. **L. Posani:** None. **S. Irfan:** None. **S.A. Siegelbaum:** None. **S. Fusi:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.05

Topic: H.06. Social Cognition

Title: Social odor coding in CA2 and its disruption in a mouse model of the 22q11.2 Deletion Syndrome

Authors: *S. BIGLER[1], S. HASSAN[2], S. A. SIEGELBAUM[3];
[1]Columbia Univ., NEW YORK, NY; [2]Neurosci., Columbia Univ., New York, NY; [3]Dept of Neurosci., Columbia Univ. Coll P & S, New York, NY

Abstract: Schizophrenia Disease (SZD) is one of many neurological disorders in which social memory is impaired; SZD patients have more difficulty recognizing previously-presented faces than their neurotypical counterparts have. At the cellular level, SZD patients show a decrease in the density of inhibitory parvalbumin (PV) interneurons in the CA2 region of the hippocampus.

Interestingly, these two phenotypes—social memory deficits and lower CA2 PV density—are reflected in a mouse model of the human 22q11.2 Deletion Syndrome (22q11.2DS), a disorder strongly linked to SZD. This mouse model (referred to as *Df(16)A*$^{+/-}$) also exhibits reduced feedforward inhibition and changes in intrinsic firing properties of CA2 pyramidal neurons, due in part to upregulation of TREK-1 K^+ current, suggesting a link between altered CA2 activity and social memory deficits in 22q11.2DS. Furthermore, silencing CA2 in wild-type mice disrupts social memory formation while restoring function to pathologically hyperpolarized CA2 neurons of *Df(16)A*$^{+/-}$ mice by administration of the TREK-1 antagonist spadin rescues their social memory deficit. We have confirmed that the population-level activity of CA2 pyramidal neurons contains sufficient information to distinguish social odors (urines) from distinct, novel conspecifics. We measured CA2's ability to encode unique social odor stimuli in a head-fixed Go/No-Go Odor Discrimination Task with 2-photon calcium imaging. Water-restricted mice were trained to lick a water port in response to one social odor (GO odor) to receive a water reward and to withhold licking to another social odor (NOGO odor) with no punishment. Here we report a profound behavioral deficit in social odor discrimination in this task comparing *Df(16)A*$^{+/-}$ mice to their neurotypical littermates as these mice fail to withhold licking to the unconditioned social odor stimulus. We are now using 2-photon imaging to assess the ability of CA2 population activity to encode and distinguish unique social odor stimuli in *Df(16)A*$^{+/-}$ versus wild-type mice, and to determine how CA2 firing and odor discrimination in the *Df(16)A*$^{+/-}$ mice is impacted by TREK-1 blockade with spadin. In this manner, we will gain further insights into the importance of CA2 in social coding and the impacts of its disruption in the 22q11.2DS mouse model.

Disclosures: S. Bigler: None. **S. Hassan:** None. **S.A. Siegelbaum:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.06

Topic: H.06. Social Cognition

Support: The Crandall-Cordero Fellowship
Peter and Carmen Lucia Buck Foundation
CT Institute for the Brain and Cognitive Sciences
UCRF
UConn SLAC
UConn IDEA Grant

Title: Dorsal and ventral hippocampus cell firing patterns in response to novel stimuli

Authors: *S. LEE, R. TROHA, Q. HU, I. STEVENSON, E. J. MARKUS;
Univ. of Connecticut, Storrs, CT

Abstract: In both humans and rats, the hippocampus is important for memory and navigation. Individual hippocampal neurons are spatially tuned, and fire in specific physical locations. Together, these "place cells" provide a representation or map of an environment. However, if the environment or behavioral context is altered, a place cell can change its firing pattern or "remap". The hippocampus is a long structure with multiple sub-regions of diverse connections. Much is known regarding place cell activity in the dorsal region of the hippocampus, since these are close to the surface of the brain and relatively accessible. Less is known about ventral hippocampal place cell activity located much deeper in the brain. We have constructed a 64-channel hyperdrive, with 16 independently moveable tetrodes targeting both dorsal and ventral regions. Firing characteristics were determined while we recorded single units simultaneously from dorsal and ventral hippocampus as rats performed a familiar/well learned task (traversing a linear track). After the hippocampal representation of the familiar task was determined, one of the two arms was rotated to a novel position. We measured how the spatial tuning of these neurons were modified when rats experienced this novel spatial environment. Similarly, we compared the hippocampal representation of the familiar task with novel social (conspecific) and olfactory (female bedding and coyote urine) experiences. We will determine dorsal and ventral hippocampal representations of a familiar maze configuration, the development of a new representation, and the degree to which the developed representation is stable. Specifically, overall firing rates and place field characteristics will be examined. Taken together, these data provide insight into the relationship between different regions of the hippocampus during the processing of a novel experience.

Disclosures: S. Lee: None. **R. Troha:** None. **Q. Hu:** None. **I. Stevenson:** None. **E.J. Markus:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.07

Topic: H.06. Social Cognition

Support: NIH F32MH120872

Title: Region-specific CaMKII-dependent synaptic plasticity is necessary for unique aspects of social recognition learning

Authors: *M. PHILLIPS, P. PARRA-BUENO, R. YASUDA;
Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: Determination of the appropriate social behavior is an essential and dynamic neural calculation in communal animals and, reflecting the complexity of the task, is dependent on proper function of numerous brain regions connected by long-range circuitry. The regions involved in social recognition memory include dorsal CA2 (dCA2), ventral CA1 (vCA1), and the prelimbic region of the medial prefrontal cortex (PL), which form a continuous, unidirectional circuit. Previous work using optogenetic and chemogenetic approaches has found that perturbation of any of these regions or their connections is sufficient to disrupt social memory. However, the mechanism of these approaches is to alter neuronal resting potential, and, due to the highly connected nature of the social memory circuit, altering the firing patterns in upstream regions alters the function of all downstream regions. Additionally, this approach does not allow us to determine which aspects of social memories each region is responsible for encoding via synaptic plasticity. These technical limitations have made it difficult to parse the unique roll of each region in social recognition memory. To address this, we utilize a photo-activatable AIP2 to inhibit CaMKII (paAIP2) in order to impair synaptic plasticity without affecting neuronal firing pattern. We plan to systematically express this genetically encoded modifier in each region (dCA2, vCA1, and PL) and wirelessly activate paAIP2 during a freely interacting social learning paradigm. By using computer vision to track and annotate a plethora of naturalistic behaviors, we will determine the necessity of region-specific synaptic plasticity to shape social behavioral choices and define each region's unique roles in these choices. Our preliminary data has found that perturbation of CaMKII in vCA1 during social learning impairs social discrimination in most interaction types, yet results in a strong in a preference for familiar conspecifics in nose-nose sniffing. While future experiments aim to compare these results to CaMKII perturbation in dCA2 and PL, these data are the first evidence for the necessity of CaMKII-dependent plasticity in vCA1 for social discrimination learning.

Disclosures: M. Phillips: None. **P. Parra-Bueno:** None. **R. Yasuda:** None.

Poster

P810. Hippocampal Cortical Social Circuits

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P810.08

Topic: H.06. Social Cognition

Support: Nancy Pritzker Funds
NIH K99 Career Development Award MH122697
NIH Grant DP2 AG067666
NIH K08 Career Development Award MH110610

Title: Serotonin modulation of a medial septal circuit tunes social memory stability

Authors: *X. WU[1], W. MORISHITA[1], K. T. BEIER[3], B. D. HEIFETS[2], R. C. MALENKA[1]; [1]Psychiatry & Behavioral Sci., Stanford Univ., Palo Alto, CA; [2]Anaesthesiology, Perioperative and Pain Med., Stanford Univ., Stanford, CA; [3]Physiol. and Biophysics, Univ. of California, Irvine, Irvine, CA

Abstract: The ability to remember familiar conspecifics, termed social memory, is critical for an animal's behaviour in its social environment. In recent years, extensive evidence supports the importance of the distinct hippocampal subregions, dorsal CA2 (dCA2) and ventral CA1 (vCA1), as well as some of their projection targets in the regulation of social memory. However, little is known about the relevant extrahippocampal input regions. Here, we present evidence that the medial septum (MS), which was not previously associated with social behaviour, sends inputs to the hippocampus that are required for social memory. Furthermore, we find that 5-HT modulation of the MS specifically and bi-directionally influences social memory formation. Using *TRAP2;Ai14* mice to take an unbiased approach for identifying dCA2 and vCA1 input regions that are not involved in object memory but are activated during social encounters, we identified elevated activity in the MS during social interactions compared to object interaction. Retrograde and anterograde tracing experiments revealed strong projections from the MS to the dCA2 and vCA1. Chemogenetic inhibition of the MS→dCA2 projection, but not the MS→vCA1 projection impaired social memory. Furthermore, optogenetic inhibition of MS→dCA2 inputs impaired the acquisition but not the expression of social memory. Strikingly, we found that chemogenetic excitation of the MS can prolong social memory. The MS contains multiple cell types but pharmacological experiments revealed a critical role only for MS glutamatergic cells in social memory formation. *Ex vivo* slice experiments demonstrated that novel social interactions increased MS→dCA2 excitatory synaptic strength. Reversing this increase with an i*n vivo* LTD induction protocol in the MS→dCA2 inputs prior or after social interaction ablated social memory. Results from a final set of experiments support a critical role for 5-HT modulation of the MS in social memory: (1) Infusion of a specific 5-HT1b receptor (R) antagonist into the MS disrupted social memory. (2) Activity of MS glutamatergic cells increased during a novel social interaction in a 5-HT1bR-dependent manner. (3) Bidirectional modulation of median raphe 5-HT inputs in the MS bidirectionally influenced social memory. (4) Infusion of a 5-HT1bR agonist into the MS prolonged social memory in wild-type mice and rescued social memory deficits in an autism-associated mouse model. Together these findings reveal a 5-HT modulated MS→dCA2 circuit that is required for the regulation of social memory.

Disclosures: X. Wu: None. **W. Morishita:** None. **K.T. Beier:** None. **B.D. Heifets:** None. **R.C. Malenka:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.01

Topic: H.12. Aging and Development

Support: NIH Grant AG060778

Title: Sex differences in the effects of age on prefrontal cortex-mediated cognition in Fischer 344 x Brown Norway F1 hybrid rats

Authors: *K. M. GONZALEZ, M. FARAJI, A. HAYMOV, V. S. KELLEY, B. SETLOW, J. L. BIZON;
Univ. of Florida, Gainesville, FL

Abstract: Sex differences in the effects of age on prefrontal cortex-mediated cognition in Fischer 344 x Brown Norway F1 hybrid rats
Katherine M. Gonzalez[2], Mojdeh Faraji[1,3], Anna Haymov[2], Vicky S. Kelley[1], Barry Setlow[2,3], Jennifer L. Bizon[1,3]
Departments of [1]Neuroscience, [2]Psychiatry, [3]McKnight Brain Institute,University of Florida, Gainesville Florida
Aging is associated with alterations in multiple aspects of prefrontal cortex (PFC)-mediated executive functions, including working memory, behavioral flexibility, and decision making. Such cognitive alterations can be modeled in rats, but almost all such work to date has been conducted exclusively in males. Taking advantage of the recent availability of aged females, we began initial evaluations of young adult (6 mo.) and aged (22 mo.) Fischer 344 x Brown Norway F1 hybrid rats of both sexes in intertemporal choice (delay discounting), working memory, probabilistic reversal learning, and progressive ratio tasks, on all of which aged males have been shown previously to differ from young adult males. In the intertemporal choice task, in which rats made choices between a small food reward delivered immediately vs. a large food reward delivered after a variable delay period (0-60 s), aged males chose the large, delayed reward more than young adult males (consistent with previous finding), whereas young and aged females did not differ. In the delayed response working memory task, in which rats had to remember the location of a lever over a 0-24 s delay period, young adults of both sexes performed more accurately than their aged counterparts. In contrast, in the probabilistic reversal learning task, in which rats had to learn to discriminate between two levers that were reinforced at different probabilities and then switched multiple times per test session, there were no age differences in the number of reversals achieved in males, whereas aged females achieved fewer reversals than young adult females. Finally, in the progressive ratio task, in which the number of lever presses required to earn a food reward increased with each successive reward earned, aged rats of both sexes made fewer lever presses than their young adult counterparts. Data from this initial experiment largely replicate previous findings of age differences in executive functions in male rats, but suggest that aging may have qualitatively or quantitatively different effects on executive functions in females. These differences further highlight the importance of using both sexes in studies employing animal models of cognitive aging.

Disclosures: K.M. Gonzalez: None. **M. Faraji:** None. **A. Haymov:** None. **V.S. Kelley:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.02

Topic: H.12. Aging and Development

Title: Acute effects of cannabis on cognition in aging

Authors: *S. ZEQUEIRA[1], A. A. GÜVENLI[2], E. C. BERTHOLD[2], M. M. BRUNER[2], J. F. DESLAURIERS[2], C. HERNANDEZ[2], C. R. MCCURDY[3], S. ABHISHEAK[4], M. FEBO[5], J. L. BIZON[7], B. SETLOW[6];
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Abstract: Legalization of recreational and medical cannabis has led to greater numbers of cannabis consumers, with individuals over the age of 65 being one of the fastest growing demographics of new users. While cannabis may offer some benefits to older adults (e.g., helping with pain, sleep, and appetite), little is known about the implications of cannabis use on cognition in this age group. Understanding the impact of cannabis on cognition in older adults is particularly critical as age-associated deficits in cognitive functions supported by both prefrontal cortex (PFC) and hippocampus are well-documented, and these same cognitive functions are impaired by acute administration of cannabis or cannabinoids (e.g., delta-9-tetrahydrocannabinol, THC) in young adults. These data suggest that cannabis use might exacerbate or even accelerate age-associated cognitive decline. The overarching goal of the current study was to use a rat model to determine how acute exposure to cannabis smoke influences cognition in young and aged subjects. In this study, the effects of acute cannabis smoke exposure were evaluated in male and female young adult (6 months) and aged (24 months) Fischer 344 x Brown Norway F1 hybrid rats using both a PFC-dependent delayed response working memory task and a hippocampal-dependent trial-unique non-match to location (TUNL) task performed in touchscreen operant chambers. A semi-randomized, within-subjects experimental design was used, such that performance of each subject was assessed under multiple dosing conditions. Specifically, cognitive performance was assessed in separate sessions immediately following exposure to smoke generated from burning 0, 3, or 5 cigarettes, with a 48-hour wash-out period between sessions. In the working memory task, cannabis smoke had opposite effects in young and aged male rats, tending to impair performance in young but enhance performance in aged. Preliminary data in females show that cannabis smoke tended to impair hippocampal-dependent TUNL performance in young rats but enhance performance in aged rats. Assessment of blood THC following cannabis smoke exposure revealed trends toward higher and more variable THC levels in aged compared to young rats. One conclusion from this study is that acute cannabis smoke exerts differential effects in aged compared to young subjects. The data also suggest that cannabis may influence cognition differently as a function of sex. Future work on the mechanisms of cannabis-mediated cognitive enhancement could clarify these

age- and sex-specific effects and better elucidate the potential utility of cannabis use in older adults as a cognitive enhancer.

Disclosures: S. Zequeira: None. **A.A. Güvenli:** None. **E.C. Berthold:** None. **M.M. Bruner:** None. **J.F. Deslauriers:** None. **C. Hernandez:** None. **C.R. McCurdy:** None. **S. Abhisheak:** None. **M. Febo:** None. **J.L. Bizon:** None. **B. Setlow:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.03

Topic: H.12. Aging and Development

Title: Maternal separation causes behavioral and neuroendocrine alteration in middle- aged female rats that are partially reversed by ovariectomy

Authors: *M. ROQUE[1,2], N. LAJUD[2], R. RUIZ[3];
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Abstract: Early life stress (ELS) in the form of maternal separation causes age- dependent behavioral and neuroendocrine alterations in male rats, however the interaction between age-dependent hormonal decline and ELS has never been studied in female rats. The aim of this work was to analyze the effects of ELS on behavior and neuroendocrine parameter in a model or post-menopause middle- aged rats. Entire litters of Sprague Dawley rats were randomly distributed into Control (undisturbed) and Maternal Separation (3 hours a day from postnatal day 1 to 14, MS180) groups. At 10 months of age, animals were ovariectomized (OVX), or remained under standard conditions (STD), and further allowed to recover for 2 months prior to the tests. When evaluating ELS and OVX as independent factors, we observed that MS180 affected cognitive performance in the Morris water maze (MWM), increased immobility time and latency to immobility in the forced swimming test (FST). MS180 increased the area under the curve in the glucose tolerance test. On the other hand, OVX affected cognitive performance in MWM and decreased struggling time in the FST. However, when analyzing the interaction of stress and OVX we observed that MS180 + OVX rats' behavior in the MWM and the FST was not significantly different from CONT + STD; however, MS180 + OVX significantly increased the area under the curve in the glucose tolerance test. In conclusion, our results indicate that ELS shows an age-dependent effect on female rats that could be partially modulated by gonadal hormones.

Disclosures: M. Roque: None. **N. Lajud:** None. **R. Ruiz:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.04

Topic: H.12. Aging and Development

Title: An ex-vivo hippocampal preparation for the study of glucocorticoid-driven serum and glucocorticoid-regulated kinase 1 (sgk1) expression

Authors: *D. R. CRAIG[1], E. M. BLALOCK[2], J. C. GANT[3], O. THIBAULT[4], Y. AL-SIRAJ[3], C. M. NORRIS[5];
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Abstract: Objective & Rationale Our objective is to elucidate the influences of stress on brain aging. Brain aging appears to be a primary risk factor for the development of prominent neurodegenerative pathologies; stress exposure has been shown to accelerate brain aging. Glucocorticoid (GC) signaling is thought to play a key role in stress' negative impact on brain function. The GC-dependent downstream effector molecule, Sgk1, is increased with both stress and aging; however, little work has been done to examine the cell-type Sgk1 splice variant expression after GC exposure in hippocampus. The hippocampus is a particularly appropriate model structure for brain aging studies based on its well-established role in cognition and memory, its manifestation of aging changes, its expression of hormone receptors, and its vulnerability to neurodegenerative disease. Here, we used the *ex vivo* hippocampal slice preparation to examine GC signaling properties in young and aged male and female rats. **Methods & Results** Hippocampal slices were maintained in oxygenated artificial cerebrospinal fluid at 32°C in an interface chamber, and incubated in either 0.1% DMSO or 3.5 µM corticosterone for 2 hours. RNA was then isolated, and concentration and integrity was assessed. cDNA was synthesized and then diluted (1:10). Real-time PCR was then performed with forward and reverse primers for Sgk1 and Sgk1.1. An increase in Sgk1 mRNA was expected with GC exposure. However, the observed increase showed only a trend towards significance. Sgk1.1 is not regulated by GCs, as it lacks a GC response element in its promotor; however, its mRNA showed a significant increase in expression. **Conclusions** This well-established slice preparation typically used for electrophysiology also is appropriate for investigating age-related GC driven Sgk1 signaling. In this preparation, GC exposure is sufficient to drive Sgk1 mRNA expression. These findings are preliminary and need to be confirmed through replication and validation of probe design. Further, adjustments to dose, duration, number of samples, as well as pre-vetting with electrophysiological techniques may be appropriate. This model may also be appropriate to:

Establish GC concentration-effect relationships, identify responding cell-types using IHC or *in situ* hybridization, and investigate pharmacologic interventions (e.g., progesterone, RU-486, and A-205). Goals in the long term: Establish hippocampal gray and white matter Sgk1 transcriptional profiles, quantify Sgk1 variant cell-type specific localization after treatment, and test Sgk1 and progesterone-based interventions in hippocampal function with stress and aging.

Disclosures: D.R. Craig: None. **E.M. Blalock:** None. **J.C. Gant:** None. **O. Thibault:** None. **Y. Al-siraj:** None. **C.M. Norris:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.05

Topic: H.12. Aging and Development

Support: This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

Title: Sleep duration and architecture in a rat model of normal cognitive aging

Authors: *C. MYRUM[1], Z. UTTKE[1], M. STELZNER[1], P. R. RAPP[2];
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Abstract: Here we examined whether cognitive outcome is related to sleep/wake physiology, as evaluated by electroencephalography (EEG) and electromyogram (EMG) analyses. The experiments capitalized on a well-established rat model of normal cognitive aging, where outbred Long-Evans rats were tested on a hippocampus-dependent version of the Morris water maze. As reported in previous studies, we observed greater inter-individual variability among aged animals, resembling the increased variability observed in aged humans. Rapid eye movement (REM) sleep, non-REM (NREM), and wake states were then manually scored based on EEG/EMG signatures for each state. The most notable preliminary findings, in groups of 4 to 6 rats, are as following: (1) Compared to young animals, aged rats spent less time in REM sleep ($p=0.01$); (2) Aged rats with intact spatial memory spent less time in REM sleep ($p=0.01$) than young rats, while REM deficits were less severe in aged rats with memory impairment ($p=0.057$); (3) Aged rats with intact spatial memory displayed more fragmented sleep than young rats ($p=0.048$); and (4) Delta power was significantly lower in aged rats compared to young animals ($p=0.04$). Given that aging in humans is generally accompanied by a decrease in the overall amount of sleep, an increase in sleep fragmentation, and a decrease in delta power during NREM sleep, the similarities with Long-Evans rats observed here may be useful for identifying the causes and/or consequences of age-related changes in sleep. These preliminary data also

suggest that aged rats with good spatial memory may be more resilient to age-related changes in sleep. Since evidence from humans indicate that sleep earlier in life may influence cognitive functioning later in life, longitudinal studies in this model and others may also therefore be warranted.

Disclosures: C. Myrum: None. **Z. Uttke:** None. **M. Stelzner:** None. **P.R. Rapp:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.06

Topic: H.12. Aging and Development

Support: NIH Grant 90065073
NIA PO1 AG09973-24

Title: Cognitive performance in aged rats associates with alter status of excitatory inputs onto interneurons in the dentate gyrus.

Authors: *C. MORENO NARANJO[1], D. SEVERIN[1], M. KOH[1], J. ZHOU[3], M. BRIDI[1], P. WORLEY[3], M. GALLAGHER[1], A. KIRKWOOD[2];
[1]Zanvyl Krieger Mind/Brain Inst., [2]Mind Brain Inst., Johns Hopkins Univ., Baltimore, MD; [3]The Solomon H. Snyder Dept. of Neurosci., Jhons Hopkins Univ., Baltimore, MD

Abstract: Aging often impairs mental abilities, including learning and memory. Recent studies have identified hyperactivity within the medial temporal lobe, in particular lateral entorhinal cortex (LEC) and the CA3/DG hippocampal subfields, as a dysfunctional condition common to cognitive impairment in aged rats and elderly humans diagnosed with mild cognitive impairment. In a previous study, we evaluated the status of synaptic inhibition and excitation in the LEC->DG pathway in a behaviorally characterized Long-Evans rat model of aging that discriminates aged impaired (AI) and aged unimpaired (AU) individuals and found that the recruitment of inhibition by LEC activation was selectively reduced in AI subjects. We are using whole-cell recording in slices to examine candidate mechanisms. First, we tested the effects of overexpressing Neuronal Pentraxin-2 (NPTX2) in LEC cells. NPTX2 is an extracellular protein released by excitatory neurons in an activity-dependent manner that is crucial for stabilizing AMPA receptors at synapses onto parvalbumin-positive inhibitory interneurons (PV-INs). Overexpression of NPTXs restored the balance of excitation and inhibition in the LEC->DG pathway of AI rats suggesting a reduced recruitment of PV-INs in AI. Next, using an AAV.mDlx.GFP transfection approach to visualize interneurons in the aged rats we found evidence that the number of excitatory LEC inputs converging onto dentate PV-INs is reduced in

AI rats. We are currently testing the hypothesis that a reduction in NPTX2 is the primary contributing factor to the altered balance of excitation/inhibition in the LEC->DG of AI rats.

Disclosures: C. Moreno Naranjo: None. **D. Severin:** None. **M. Koh:** None. **J. Zhou:** None. **M. Bridi:** None. **P. Worley:** None. **M. Gallagher:** None. **A. Kirkwood:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.07

Topic: H.12. Aging and Development

Support: 5R56MH095980-07

Title: Synaptic culprits underlying cognitive decline in the aged brain

Authors: *T. LIU[1], V. MATHESH[1], J. FALCO[1], M. MOTA[1], K. M. HARRIS[2], L. M. KIRK[2]; [2]Ctr. for Learning and Memory, [1]Univ. of Texas at Austin, Austin, TX

Abstract: Age-related cognitive decline is developing into one of the biggest challenges in modern society. As the population ages, our health system is becoming stressed. During normative aging, cognitive changes are not associated with neuron loss; instead, changes in synapse structure have become the focus of inquiry. Aged Long Evans rats (25-27 months) were categorized as aged learning impaired (AI) or aged learning unimpaired (AU) compared to young adult controls (YA; 3-6 months) using the Morris water maze (MWM). Serial section electron microscopy of dendrites and their synapses from hippocampal area CA1 revealed that AI rats had increased excitatory synapse size and fewer inhibitory synapses onto CA1 dendrites. Thus, there was a dramatic increase in the excitatory/ inhibitory ratio along dendrites from AI rats compared to YA controls, providing synaptic evidence for age-related hyperexcitability underlying cognitive decline. Dendrites from AU rats are being analyzed to determine whether these changes are ubiquitous with aging or if they are specific to age-related learning impairments. Presynaptic components are also being analyzed. These data provide new clues about the synaptic culprits that undermine brain reserve and resilience in aged animals with cognitive decline.

Disclosures: T. Liu: None. **V. Mathesh:** None. **J. Falco:** None. **M. Mota:** None. **K.M. Harris:** None. **L.M. Kirk:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.08

Topic: H.12. Aging and Development

Title: Changes in hypoxia-inducible factor expression in aged hippocampus

Authors: *B. SNYDER, H.-K. WU, B. TILLMAN, T. F. FLOYD;
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Abstract: The hippocampus (HIPP) is susceptible to hypoxic insults and conditions which confer hypoxia increase during aging, potentially underlying HIPP vulnerability to acute bouts of hypoxia (i.e., stroke or surgery). Alpha isoforms of hypoxia-inducible factors (HIFa) initiate protective hypoxic responses and are dysregulated in aged cortex, but their action within the HIPP has not been fully delineated. We hypothesize that HIFa protein expression is impaired in aged hippocampus and coupled with deficient transcription of downstream genes during acute hypoxia.

Mice, aged 3, 9, 18, & 24 months (mo), were exposed to normoxia (21% oxygen [O_2]) or hypoxia (8%) for 3 hours. Coronal sections containing the hippocampus were immediately collected and flash frozen, sliced on a cryostat into 10 um slices, and mounted on slides. Protein expression of HIFa was assayed by immunohistochemistry (IHC). *In situ* hybridization (ISH) was used to probe mRNA of 10 genes in the HIF pathway: HIF1a (*hif1a*), HIF2a (*epas1*), erythropoietin (*epo*), vascular endothelial growth factor (*vegf*), glucose transporter (*glut1*), lactose dehydrogenase-A (*ldha*), phosphoglycerate kinase1 (*pgk1*), & pyruvate dehydrogenase kinase 1 (*pdk1*). Fluorescence intensity of IHC was captured on a Leica DMI8. ISH slides were scanned on a Zeiss Axioscan. Intensity or the number of ISH spots per HIPP was processed in FIJI. Results were normalized by HIPP area and ANOVAs with Bonferonni post-hoc comparisons performed in JMP 14.

Basal expression of HIF1a protein was elevated within the HIPP of aged mice and stabilization decreased with age under 8% O_2. A similar pattern was observed in *hif1a* mRNA. Only the 24 mo group exhibited a basal elevation of HIF2a protein. Basal expression of *epo, vegf, mct1, and mct4* mRNA was elevated in aged HIPP. Basal expression of *glut1, ldha, pgk1,* & *pdk1* mRNA was lower in aged HIPP. The 3 mo transcriptional changes observed during 8% O_2 showed trends for reversal in aged HIPP.

The age-related elevation of HIFa and changes in gene expression suggests mild hypoxia exists during aging. Genes involved in metabolism maintain energy substrates for neuronal signaling during hypoxia. The changes observed here indicate there may be deficits in metabolic genes necessary to maintain cognitive processes during aging-related hypoxia. Future studies will focus upon potential points of failure in the regulation of HIFa in the HIPP during aging, as well as the contribution of metabolic and trophic HIF-genes to synaptic plasticity in the HIPP.

Disclosures: B. Snyder: None. **H. Wu:** None. **B. Tillman:** None. **T.F. Floyd:** None.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.09

Topic: H.12. Aging and Development

Support: CPRIT Research Training Award RP170345
CPRIT RP180055
NCI R01 CA224675
The Quincy and Estine Lee Endowment Fund

Title: Vortioxetine, a multimodal antidepressant, reverses cognitive impairments associated with androgen deprivation therapy using the gonadotropin-releasing hormone antagonist, degarelix in a middle-aged rodent model

Authors: *A. VAIANA[1,2,3], J. GELFOND[4], T. JOHNSON-PAIS[3,5], R. LEACH[3,6], C. RAMAMURTHY[3,7], I. THOMPSON[5,8], D. MORILAK[1,2,3,9];
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Abstract: Androgen deprivation therapy (ADT) is a mainstay treatment for late-stage prostate cancer. However, ADT induces cognitive impairment in over half of patients and increases the likelihood of dementia and Alzheimer's disease. These impairments significantly reduce quality of life for cancer survivors. Unfortunately, there are few treatment options available to patients to prevent or reverse these deficits. Previously we demonstrated in a rodent model of ADT that young adult male rats exhibit deficits in attentional set-shifting and spatial learning after surgical castration. These cognitive processes, mediated by the medial prefrontal cortex (mPFC) and hippocampus, are also disrupted in ADT patients. Vortioxetine is a multi-modal antidepressant which improves cognition in depressed patients, and we hypothesized that it may improve cognition after ADT. We found that chronic dietary vortioxetine (28 mg/kg/day, 21 days) was able to reverse the deficits in set-shifting and spatial learning in castrated animals. To increase the translational relevance of our model of ADT, the current study investigates cognitive impairments induced by chemical castration with the gonadotropin releasing hormone antagonist, degarelix. Further, the average age of onset in prostate cancer patients is approximately 65, so we used middle-aged 12-month-old rats, an age at which mild age-related cognitive impairments emerge. Young and middle-aged rats were injected with 3 mg/kg degarelix in 5% mannitol. Controls received a vehicle injection of 5% mannitol. Animals were then fed vortioxetine or control chow through completion of the novel object location (NOL) test

and the attentional set-shifting test (AST). There were no baseline differences between young and middle-aged control rats in either test (p=0.85). ADT trended toward impaired visuospatial memory and set-shifting at both ages (NOL: young p=0.07/aged p=0.15; AST: young p=0.08/aged p=0.16). Vortioxetine trends towards a reversal of impairment on NOL and on AST (NOL: young p=0.08/aged p=0.19; AST: young p=0.15/aged p=0.16). More animals will be added to these groups to achieve sufficient power for analysis. Experiments are underway to investigate changes in functional connectivity of the mPFC and dHipp in the aged population by measuring changes in evoked local field potentials. Our early results indicate that vortioxetine may reverse ADT-induced cognitive decline in middle-aged animals with chemical castration, indicating that it could be useful in mitigating cognitive changes in older men treated for prostate cancer.

Disclosures: A. Vaiana: None. **J. Gelfond:** None. **T. Johnson-Pais:** None. **R. Leach:** None. **C. Ramamurthy:** None. **I. Thompson:** None. **D. Morilak:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); H. Lundbeck, Copenhagen, Denmark.

Poster

P893. Learning and Memory in Aging: Animal Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P893.10

Topic: H.05. Working Memory

Title: Role of RalBP1 in Cognitive Dysfunction and Neuronal Aging in mouse model

Authors: *S. P. SINGH[1], H. MORTON[1], S. KSHIRSAGAR[2], C. BOSE[2], A. HINDLE[2], N. A. SAWANT[2], L. E. BUNQUIN[2], M. VIJAYAN[2], P. T. PALADE[3], J. J. LAWRENCE[4], P. REDDY[5], S. AWASTHI[5];
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Abstract: Background: The purpose of our study is to understand the role of RALBP1 in oxidative and mitochondrial dysfunction in relation to aging. The Ralbp1 gene encodes the 76 kDa protein Rlip (aka RLIP76), is transcriptionally regulated by EP300, a CREB-binding protein that is important for the synaptic plasticity, memory, long-term potentiation and stress-protection in neurons. Rlip is a stress-responsive/protective transporter of glutathione conjugates (GS-E) and xenobiotic toxins. **Methods:** A total of 10 male 10-month-old mice (5 WT and 5 $Rlip^{+/-}$) were tested for neurocognitive function using open field maze, Morris Water Maze, Y-maze, and rotarod instruments. Brain tissues obtained from cohorts of wild-type and $Rlip^{+/-}$ mice were analyzed for oxidative stress markers, expression of genes that regulate mitochondrial fission/fusion, and synaptic function. **Results:** We evaluated whether Rlip deficiency in mice

may develop AD like loss of cognitive function and mitochondrial dysfunction. Behavioral, basic motor and sensory function tests in $Rlip^{+/-}$ mice revealed cognitive neuropsychiatric impairements. Our studies also revealed a significant increase in the levels of oxidative stress markers and alterations in expression of genes and protein involved in mitochondrial biogenesis in brain tissues of these mice. **Conclusions:** Present studies have shown that partial suppression of Rlip protein associated increase in oxidative stress and the associated mitochondrial dysfunction might be implicated in the development of AD processes. Our findings also indicate a potential role of an unknown Rlip-NRF2 interaction that is impaired in AD. Therefore, the restoration of Rlip activity and endogenous cytoprotective mechanisms may be a safe and effective strategy to protect against AD.

Disclosures: S.P. Singh: None. **H. Morton:** None. **S. Kshirsagar:** None. **C. Bose:** None. **A. Hindle:** None. **N.A. Sawant:** None. **L.E. Bunquin:** None. **M. Vijayan:** None. **P.T. Palade:** None. **J.J. Lawrence:** None. **P. Reddy:** None. **S. Awasthi:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: R35GM119831

Title: Candidate enhancers in the psychiatric-associated third intron of CACNA1C show activity in P28 mouse brain

Authors: T. WARREN[1], L. SU-FEHER[2], E. CASTILLO[3], J. L. HAIGH[4], K. CICHEWICZ[1], J. T. LAMBERT[5], *A. NORD[3];
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Abstract: *Cis*-regulatory elements such as enhancers play critical roles in the spatiotemporal regulation of gene expression in the developing brain. Genome-wide association studies (GWAS) have consistently linked a large block in the third intron of the gene *CACNA1C* with risk for psychiatric disorders, including schizophrenia and bipolar disorder. We adapted an enhancer reporter assay known as STARR-seq for delivery into the mouse brain using recombinant adeno-associated virus (rAAV) as an expression vector to test candidate enhancers for activity in postnatal mouse brain. Using this method, we identified three strong enhancers in the risk region of *CACNA1C* in postnatal day (P) 7 mouse brain. Follow-up testing of our top identified enhancers in *CACNA1C* shows they continue to drive strong expression of a reporter gene in mouse brain through P28. This approach enables *in vivo* testing of disease-associated cis-

regulatory elements across multiple stages of development. Such functional examination of enhancers will be critical toward dissecting the regulatory activity of enhancers in the brain and how enhancer sequence variation contributes to brain development and neurological disorders.

Disclosures: T. Warren: None. **L. Su-Feher:** None. **E. Castillo:** None. **J.L. Haigh:** None. **K. Cichewicz:** None. **J.T. Lambert:** None. **A. Nord:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA U01DA043098
Office of Naval Research (ONR) 00014-19-1-2149
The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium

Title: Sex-differences in selectively bred high- and low-responder rats: spatial gene expression in the dorsal hippocampus

Authors: *M. WASELUS, E. HEBDA-BAUER, M. DAI, F. MENG, H. AKIL, S. J. WATSON, Jr.;
Mol. Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Historically, our laboratory has used radioactive in situ hybridization to localize and quantify differences in gene expression in various brain regions. Our selectively-bred lines of high- and low-responder rats (bHR and bLR, respectively) are advantageous in that we can use this model to study two “extreme” behavioral phenotypes (e.g., differences in locomotor activity in a novel environment, propensity to self-administer drugs of abuse, and anxiety-like behaviors). In situ hybridization has been used to measure gene expression in the brains of these rats which can then be mapped back on to behavioral differences. Unfortunately, traditionally used radioactive in situ hybridization studies are labor-intensive and costly, with only a finite amount of material available from a single subject thus limiting the number of genes examined. Recently, spatial transcriptomics, most recently the Visium Spatial Gene Expression solution developed by 10X Genomics (Pleasanton, CA), allows RNA-seq on a small region of interest within tissue sections, reducing the hands-on processing time and increasing the number of genes examined in a single study while retaining spatial resolution. Brains from the 68^{th} generation of our selective breeding colony were used to examine both bHR/bLR- and sex differences in gene expression in the dorsal hippocampus of adult rats. One section per “condition” (bred line x sex) was collected from a bHR and bLR family per slide, allowing each slide to function as its own

independent study. Adjacent sections were collected on a second slide to serve as a technical replicate. A second bHR and bLR family was used to examine bHR/bLR and sex differences, serving as a biological replicate. Our initial results indicate that all Visium samples were of high quality for use in downstream analyses. We first wanted to demonstrate that known markers of sex differences were different male and female rat brain, regardless of breeding background. To that end, we found two examples of Y-linked genes (i.e., Eif2s3y and Kdm5d) are expressed in the brains of male, but not female, rats. Furthermore, specific genes of interest are differentially expressed in the brains of adult bHR and bLR rats, consistent with other ongoing internal studies. Our conclusions thus far indicate that in our hands, Visium has the potential to be a powerful tool for elucidating differences in gene expression between not only male and female rats, but also selectively bred bHR and bLR rats. Further studies will allow us to combine single cell data generated by our group with spatial datasets to determine the precise localization of gene expression differences within larger brain structures.

Disclosures: M. Waselus: None. **E. Hebda-Bauer:** None. **M. Dai:** None. **F. Meng:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Molecular atlassing with MERSCOPE: A new spatial transcriptomics technique accurately reveals the organization of the mouse brain transcriptome

Authors: *G. EMANUEL, J. HE, J. HE;
Vizgen, Cambridge, MA

Abstract: Biological systems are comprised of numerous cell types, intricately organized to form functional tissues and organs. Building molecular atlases to fully understand the structure and function of each cell within the brain is now a key aspect of neuroscience research. Atlas initiatives using single-cell RNA sequencing can characterize cell types based on their RNA expression profiles. However, the tissue organization is lost when cells are dissociated for single-cell sequencing, making it difficult to study how the cellular heterogeneity is contributing to the function of the tissue. Furthermore, accurately characterizing each cell within the brain is challenging due to the low expression of many functionally important genes such as nonsensory G-Protein coupled receptors (GPCRs). GPCRs mediate signaling and may play vital roles in brain aging and neurodegenerative disorders but are difficult to analyze. Thus, the ability to completely spatially profile GPCR expression across the brain with cellular context can enable a deeper understanding of brain tissue. A spatial transcriptomics technique with high detection

efficiency and single molecule resolution is required to build a complete molecular atlas. Vizgen's in situ genomics platform MERSCOPE™ enables the direct profiling of the spatial organization of intact tissue with subcellular resolution. MERSCOPE is built on multiplexed error robust in situ hybridization (MERFISH) technology that uses combinatorial labeling, sequential imaging, and error-robust barcoding to provide the highest detection efficiency and resolution available for spatial genomics. In a single experiment, hundreds of thousands of cells can be spatially profiled with high accuracy and reproducibility. To demonstrate the power of MERSCOPE, we mapped 483 genes across three different positions of the mouse brain with three biological replicate tissue slices per position. We constructed a panel that includes canonical cell type markers and GPCRs to spatially profile gene expression across the brain. Among the approximately half-billion total transcripts detected, we successfully located notably low expressing nonsensory GPCRs such as Oxtr, Tshr, and Insr. We also demonstrated a strong correlation (r=0.88) between the number of transcripts detected to bulk RNA sequencing. From MERSCOPE's cell segmentation, we clustered cells by gene expression, identified different cell types, and mapped their spatial organization. The MERFISH mouse brain receptor map establishes MERSCOPE as a leading technique for molecular atlassing, empowering scientists to find greater insights into healthy versus diseased tissue.

Disclosures: G. Emanuel: None. **J. He:** None. **J. He:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH 5RO1NS073129
NIH 5RO1DA036913
NIH RF1MH114132
NIH U01MH109113
NIH R01MH113005
NIH R01LM012736
NIH U19MH114821

Title: Spatial distribution of transcriptomic neuronal types across the whole cortex determined by in situ sequencing

Authors: *X. CHEN[1], S. FISCHER[2], J. GILLIS[2], A. M. ZADOR[2];
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Abstract: The mammalian cortex is composed of myriad long-range projection neurons that enable communication within the cortex and between the cortex and other brain structures. Previous studies using single-cell RNAseq have revealed tremendous diversity in the gene expression of cortical projection neurons, but how these transcriptomic types of neurons are distributed across the whole cortex is largely unknown. Here we determined the transcriptomic identities of 1.2 million neurons across a mouse hemi-brain and mapped their spatial distribution using a high-throughput in situ sequencing pipeline. Consistent with previous studies, known transcriptomic subclasses of neurons are differentially distributed within the isocortex, across cortex-like structures, and across non-cortical brain structures. Beyond the subclass-level divisions, we found gradients of gene expression that map onto spatial gradients within the isocortex. Our approach is high throughput and can be conveniently scaled up to interrogate brain-wide cell type distribution across development and aging, across genotypes, across species, and across individual animals. Furthermore, our data provide a foundation for future interrogation of cell type long-range connectivity at a whole-brain scale.

Disclosures: X. Chen: None. **S. Fischer:** None. **J. Gillis:** None. **A.M. Zador:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cajal Neuroscience. F. Consulting Fees (e.g., advisory boards); Cajal Neuroscience.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NHGRI Grant 1R43HG010603-01A1

Title: Single nucleotide resolution profiling techniques based on eCLIP reveal functional neuronal miRNA target sites and their regulatory features

Authors: *S. A. MANAKOV, D. C. COX, K. SHEN, A. A. SHISHKIN, K. B. CHAPMAN; Eclipse Bioinnovations, San Diego, CA

Abstract: Techniques that can identify functional miRNA sites and their regulatory features are important for characterizing pathology of neuronal health, plasticity, and development of therapeutics. Methods to experimentally read out miRNA targets in the form of miRNA:target chimeric reads exist, but their application was limited due to complexity of library preparation and low rate of chimeras. N6-methyladenosine (m6A) modification in first position at miRNA target sites (t1-A) reduces efficacy of miRNA binding raising possibility that m6A can derepress miRNA sites, but modifications in the genomic environment of miRNA target sites were not yet

characterized with high resolution since single nucleotide profiling of m6A is challenging. We developed miR-eCLIP, an AGO2 eCLIP assay with chimeric ligation, and combined it with two different enrichment strategies to enrich for chimeric reads specific to a gene of interest or an miRNA of interest. We demonstrate the utility of gene specific enrichment by enriching chimeric reads for the Amyloid Beta Precursor Protein (APP) gene in HEK293xT cells. This methodology revealed five miRNA target sites in the 3'UTR of the APP transcript, three of which were co-targeted by 3 to 5 seed family member miRNAs. Co-targeting of the same seed site on the mRNA transcript by multiple miRNA family members is consistent with previous reports that knock-outs of individual miRNAs often has only mild effects on gene expression.To demonstrate the utility of miRNA-specific probe-based enrichment, miR-eCLIP with enrichment for miR-124 chimeric reads was conducted in miR-124 transfected cells. This technique identified hundreds of functional targets and RNAseq conducted in parallel confirmed that these targets have reduced expression levels. Moreover, targets were identified quantitatively, with greater number of chimeric reads associated with greater repression. Finally, we also developed m6A-eCLIP where m6A antibodies are UV crosslinked to RNA, and m6A is read out with a single nucleotide resolution by identifying reverse transcriptase termination at crosslinks. We also applied m6A-eCLIP to HEK293xT RNA and generated a map of over 200,000 m6A sites. Analysis of the m6A map showed that this modification was depleted in a 10 nt window around the functional miR-124 sites, suggesting that m6A modifications in close proximity to miRNA target sites may interfere with miRNA binding. In conclusion, we show that eCLIP techniques are suitable to study targets of neuronal miRNAs and genes on examples of miR-124 and APP gene. We believe these methodologies will be a useful tools for researchers studying neuronal miRNAs, their regulation and function.

Disclosures: S.A. Manakov: None. **D.C. Cox:** None. **K. Shen:** None. **A.A. Shishkin:** None. **K.B. Chapman:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Human AD Center Grant
NIH Mouse Brain Atlas Grant

Title: Spatial transcriptomics at scale: mapping mammalian CNS cell types

Authors: B. LONG[1], Z. MALTZER[2], M. HUPP[2], J. CAMPOS[2], J. VILLANUEVA[2], M. KUNST[2], *J. CLOSE[1], J. WATERS[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Insitute for Brain Sci., Seattle, WA

Abstract: Spatial profiling of gene expression is an essential complement to single cell transcriptomics methods, providing an unbiased method for building spatial maps that inform on cell type locations, proportions and interactions. These maps will ultimately be critical in deciphering brain circuitry and function from development through neurodegenerative disease. We have built a spatial transcriptomics data production pipeline to perform multiplex, error-robust fluorescence in situ hybridization (MERFISH) and related techniques at scale in multiple species, which will make profiling of multiple brain areas feasible. Here we describe our pipeline structure, and the resulting cell type mapping results obtained from human middle temporal gyrus as well as mouse primary visual cortex.

Disclosures: B. Long: None. **Z. Maltzer:** None. **M. Hupp:** None. **J. Campos:** None. **J. Villanueva:** None. **M. Kunst:** None. **J. Close:** None. **J. Waters:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Paul G. Allen
NIH Grant RF1MH121274

Title: Enhancer AAVs for targeting layer 6 IT Car3 type neurons and Claustrum

Authors: *E. L. MORIN, A. E. SEDENO-CORTES, C. TRADER, J. GIBSON, J. L. BENDRICK, M. TAORMINA, R. GALA, S. YAO, J. WATERS, H. ZENG, B. TASIC, T. L. DAIGLE;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Enhancer-based AAVs for targeting at the cell class-, subclass-, or type-specific levels can be combined with the evolved, blood-brain barrier-crossing PHP.eb capsid for whole-brain labeling with relative ease in mouse (Graybuck and Daigle, et al., Neuron 2021 and Mich et al., Cell Reports 2021). Here, we describe the generation and characterization of a collection of enhancer-based AAVs targeting a distinct, transcriptomically-defined type of intratelencephalic (IT) neuron: Layer 6 (L6) IT Car3. The cell bodies of these deep layer projection neurons reside in many cortical areas as well as in claustrum, an enigmatic region reciprocally connected to all cortical regions and thought to coordinate or conduct this connectivity. We identified putative enhancers for this cell type via the integrative analysis of single cell (sc) RNA-seq with scATAC-seq data from mouse primary visual cortex and cloned these into viral vectors expressing recombinases or fluorescent reporters. Individual viruses were delivered into the retroorbital sinus of Ai193 transgenic mouse, a TIGRE3.0 Cre- and Flp-dependent cell-filling

fluorophore reporter, or into C57Bl/6J mice. Viral labeling patterns were examined by serial two-photon tomography (TissueCyte) four weeks post-infection. Image datasets were registered to the Allen Mouse Common Coordinate Framework v3.0, and the fluorescent signal was segmented to construct regional labeling densities and confirm viral labeling in L6 and claustrum. Additionally, computational methods for two-color quantification of these cells are being developed, by means of a custom Unet segmentation algorithm, which will enable detailed examination of viral labeling patterns at single-cell resolution across the entire brain. Additional molecular validation (scRNA-seq profiling and multiplex FISH) of virally-labeled cells located in the primary visual cortex and claustrum will be performed to confirm cell type identity and the completeness of labeling. This work establishes a level of systematic characterization of enhancer-based viral genetic tools that did not previously exist and is critical for scaling the development of viral tools to access diverse brain-wide neuronal cell types.

Disclosures: E.L. Morin: None. **A.E. Sedeno-Cortes:** None. **C. Trader:** None. **J. Gibson:** None. **J.L. Bendrick:** None. **M. Taormina:** None. **R. Gala:** None. **S. Yao:** None. **J. Waters:** None. **H. Zeng:** None. **B. Tasic:** None. **T.L. Daigle:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Transcriptomic analysis of neuronal sub-populations using RNAscope™ guided Digital Spatial Profiling

Authors: *A. VANSCHOIACK[1], E. A. BROWN[1], A. DIKSHIT[2], S. BASAK[2], L. PAN[1], K. FUHRMAN[1], Y. LIANG[1], J. BEECHEM[1];
[1]NanoString Technologies, Seattle, WA; [2]Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA

Abstract: Defining the molecular architecture of gene expression within the brain is critical for understanding normal brain function and for developing and testing new hypotheses toward understanding and treating neurological disorders and disease. Substantial advances have been made within the last decade to enable detailed molecular characterization of neuronal populations within the mouse and human brain but have been limited by low-plex (IHC/IF/ISH) and/or low throughput (LCM, IMC) or lacking spatial resolution (single cell RNAseq). Here, we demonstrate a combinatorial approach of using RNAscope ISH-IF to guide spatial whole-transcriptomic profiling of excitatory and inhibitory neuronal populations within mouse coronal FFPE sections. Spatial transcriptomic profiling of 5um FFPE sections from four C57BL/6 mice was performed using NanoString's Mouse Whole Transcriptome Assay and the Co-detection

workflow from ACD, a Bio-Techne brand, for simultaneous RNA and protein detection, with NeuN protein visualized in combination with *Vglut1*, *Vglut2*, and *Vgat* mRNA to identify excitatory and inhibitory neuron populations. The dual ISH-IF images were captured with the GeoMx Digital Spatial Profiler (DSP) and used to identify regions in the cortex, hippocampus, and striatum within a single coronal section. Using the unique ability of GeoMx to segment and independently profile cell populations using fluorescence guided morphology, we profiled and analyzed excitatory and inhibitory neurons separately in each identified ROI, quantitating expression of the whole transcriptome in neuronal subpopulations throughout the brain with average detection in each population ranging from 5000-8000+ genes. High concordance was observed between gene expression detected by GeoMx and representative genes quantified by RNAScope, and data were also benchmarked against the Allen Brain Atlas resources as independent validation of both cell-type specific (Cell Types scRNA-seq data) and spatial (Mouse Atlas ISH database) gene expression. In conclusion, we have established a workflow enabling whole transcriptome data from spatially mapped neuron populations within healthy mice and demonstrate high precision and accuracy of the approach. We demonstrate gene expression differences in excitatory and inhibitory neurons based on spatial localization, highlighting the critical importance of spatial context in transcriptomic analysis. These data and workflow can be used as standards to inform future profiling studies in mouse models of neurological disorders and disease.

Disclosures: **A. VanSchoiack:** A. Employment/Salary (full or part-time):; NanoString Technologies. **E.A. Brown:** A. Employment/Salary (full or part-time):; NanoString Technologies. **A. Dikshit:** A. Employment/Salary (full or part-time):; Advanced Cell Diagnostics, a Bio-Techne brand. **S. Basak:** A. Employment/Salary (full or part-time):; Advanced Cell Diagnostics, a Bio-Techne brand. **L. Pan:** A. Employment/Salary (full or part-time):; NanoString Technologies. **K. Fuhrman:** A. Employment/Salary (full or part-time):; NanoString Technologies. **Y. Liang:** A. Employment/Salary (full or part-time):; NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.09

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant AG071587
NIH Grant NS114130
NIH Grant NS099474

Title: Concomitant transcriptomic and proteomic profiling using TurboID proximity labeling

Authors: *C. C. RAMELOW[1], S. SUNNA[1], S. RAYAPROLU[1], M. SAMPSON[2], N. T. SEYFRIED[3], S. SLOAN[2], S. RANGARAJU[1];
[1]Neurol., [2]Human Genet., [3]Biochem., Emory Univ., Atlanta, GA

Abstract: Biological and therapeutically relevant insights into cellular disease mechanisms of neurons and glia can be obtained by complimentary molecular profiling of the transcriptome and proteome of these cells. While mutually exclusive transcriptomic and proteomic pipelines are available, the information surveyed is often from different cells leading to poor correlation between the transcriptome and proteome. Thus, our overall goal was to develop a method for concomitant cell type-specific analyses of RNA and protein. To accomplish this, we utilized a proximity-labeling strategy that uses the biotin ligase, TurboID, to efficiently label the proteome, in a mouse microglial BV2 cell line. For proof-of-concept *in vitro* experiments, we created a BV2-TurboID cell line that stably expresses TurboID fused to a nuclear export sequence. Biotin treatment of BV2-TurboID cells resulted in robust biotinylation of the cellular proteome as confirmed by Western blot and by label-free quantitation mass spectrometry (LFQ-MS) of biotinylated proteins enriched with streptavidin beads. LFQ-MS revealed that TurboID biotinylates several RNA-binding proteins (RBPs) including ribosomal units, suggesting that transcripts associated with RBPs may also be pulled down simultaneously with proteins. To test this hypothesis, we homogenized BV2-Turbo and control BV2 cells, enriched biotinylated proteins with streptavidin beads while maintaining RNA-protein interactions, and then eluted RNA. Quality control studies showed negligible mRNA from streptavidin pulldowns from control BV2 cells, while BV2-Turbo pulldowns had larger mRNA yields with high quality. NanoString neuroinflammatory profiling (800 genes) of whole cell RNA from control BV2 and BV2-Turbo cells and streptavidin pulldowns from both cell types were performed. We observed a 23-fold higher mRNA yield in the BV2-Turbo pulldowns compared to control BV2 cells for 550 genes included in the analyses. Transcript abundances from whole cell RNA and BV2-Turbo pulldowns were highly comparable (R^2=0.97 and no differentially expressed genes) with equivalent abundance of microglial genes (e.g., *Spp1* and *Apoe*) suggesting a faithful transcriptome capture. Since we have optimized the pipeline for this method, our novel TurboID proximity labeling approach can simultaneously capture cell type-specific transcriptomes and proteomes. We are now validating this method in other cell types using RNA-seq and MS approaches. Once validated, this concurrent RNA and protein profiling approach can be applied to *in vivo* and *ex vivo* model systems to investigate the distinct roles brain cell types play in development, aging, and disease.

Disclosures: C.C. Ramelow: None. **S. Sunna:** None. **S. Rayaprolu:** None. **M. Sampson:** None. **N.T. Seyfried:** None. **S. Sloan:** None. **S. Rangaraju:** None.

Poster

P914. Genomic and Transcriptomic Techniques I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P914.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: UG3MH120094 (WRS)
DP2MH113095 (WRS)

Title: Transcriptional and Anatomical Diversity of Medium Spiny Neurons in the Primate Striatum

Authors: *J. HE[1], M. KLEYMAN[5], J. CHEN[1], A. ALIKAYA[1], K. M. ROTHENHOEFER[1], B. E. OZTURK[2], M. WIRTHLIN[5], A. C. BOSTAN[3], K. FISH[4], L. BYRNE[2], A. R. PFENNING[5], W. R. STAUFFER[1];
[1]Neurobio. Dept., [2]Ophthalmology Dept., [4]Psychiatry Dept., [3]Univ. of Pittsburgh, Pittsburgh, PA; [5]Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The striatum is the interface between dopamine reward signals and cortico-basal ganglia circuits. It is critical for learning, cognition, movement, reward, and emotional responses. The striatum can be anatomically and functionally divided into the caudate and putamen, including striosome and matrix compartments, and the ventral striatum, including nucleus accumbens (NAc), olfactory tubercle, and interface islands. Medium spiny neurons (MSNs) constitute the vast majority of striatal neurons and are traditionally classified as direct- or indirect-pathway neurons, though further diversity has been shown in rodents. Here, we profiled MSN-diversity in non-human primates (NHPs) striatum. Using a combination of scRNA-seq and FISH we defined molecularly distinct MSN subtypes including (1) dorsal striatum MSN subtypes associated with striosome and matrix compartments, (2) ventral striatum subtypes associated with the nucleus accumbens shell and olfactory tubercle, and (3) an MSN-like cell type restricted to m-opioid receptor rich islands in the ventral striatum. We show that there are conserved patterns of continuous variation throughout the striatum, but that each transition between MSN subtypes is characterized by discontinuities in gene expression. Archetypal analysis revealed further axes of variation by revealing a significant caudate signal characterized by the expression of *OPRM1*, and a unique, *TAC3*-positive NAc shell D1-MSN archetype. Together, these results constitute a 'roadmap' for investigating cell type-specific information processing in the striatum.

Disclosures: J. He: None. **M. Kleyman:** None. **J. Chen:** None. **A. Alikaya:** None. **K.M. Rothenhoefer:** None. **B.E. Ozturk:** None. **M. Wirthlin:** None. **A.C. Bostan:** None. **K. Fish:** None. **L. Byrne:** None. **A.R. Pfenning:** None. **W.R. Stauffer:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.01

Topic: A.07. Developmental Disorders

Title: Foxr1 is necessary for survival and proper brain development

Authors: *H. WAXMAN, A. MOTA, G. LAGANI, U. BEFFERT, A. HO;
Boston Univ., Boston, MA

Abstract: In the developing brain, complex and precisely timed sequences of cellular signaling are mediated by transcription factors (TFs) which drive expression of critical proteins. As such, understanding the identities of these TFs and their transcriptional networks will be paramount to understanding the etiology of neurodevelopmental disorders. The NIH Undiagnosed Disease Network identified a rare human missense mutation in *FOXR1* linked to an individual presenting with postnatal microcephaly, progressive brain atrophy, and global developmental delays. FOXR1 belongs to a large family of TFs known as *forkhead*, named for the abnormal head shape in *Drosophila* mutants. While the FOX family is relatively understudied, a subset have been demonstrated to play essential roles during brain development including FOXG1, FOXC2, FOXL2, FOXP1, and FOXP2. To date, the function of FOXR1 is unknown. Human FOXR1 shares 66% amino acid sequence identity with its mouse homologue. Using primers specific for mouse *foxr1*, we demonstrated by qPCR that *foxr1* mRNA was detected in several tissues including heart, liver, lung, and elevated expression in the brain at embryonic day 17. To better understand the role of FOXR1 in mammalian brain development, we analyzed newly developed mice that lack the *foxr1* gene, generated with the CRISPR/Cas9 gene editing system. Single guide RNAs were designed to target intron one and the majority of exon 4, thus destroying the exon 4 splice acceptor. This leads to a deletion of exons 2, 3 and majority of exon 4, resulting in a 976 bp deletion. We found that mice lacking *foxr1* displayed a severe survival deficit, while surviving newborn *foxr1* knockout mice had reduced body weight. Further examination of newborn *foxr1* knockout brains revealed a decrease in cortical thickness and enlarged ventricles compared to littermate wild-type mice, suggesting that loss of *foxr1* leads to atypical brain development. Combined, these results suggest *foxr1* is necessary for normal brain development. Future studies will further elucidate the specific temporal, cellular, and anatomical expression profile of *foxr1* expression and define foxr1 transcriptomic regulation associated with brain development.

Disclosures: H. Waxman: None. **G. Lagani:** None. **U. Beffert:** None. **A. Ho:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.02

Topic: A.07. Developmental Disorders

Support: NIH Grant AA026756
NIH Grant F30AA027698

Title: Differences in post-stroke immune response outcomes in adult rats prenatally exposed to alcohol

Authors: *M. R. PINSON, S. BAKE, A. M. ADAMS, S. PANDEY, F. SOHRABJI, R. C. MIRANDA;
Neurosci. and Exptl. Therapeut., Texas A&M Univ. Col. of Med., Bryan, TX

Abstract: Prenatal alcohol exposure (PAE) can result in increased risk for cardiovascular disease and peripheral and central immune system dysfunction which may contribute to negative outcomes post-stroke. To test this hypothesis, timed-pregnant Sprague-Dawley rats were exposed to binge-like episodic ethanol vapor or ambient air during the latter half of gestation. Pups were allowed to age to 12 mo at which point they underwent surgeries for middle cerebral artery occlusion (MCAO), a model for cerebrovascular stroke. At 2 days post-MCAO, animals were euthanized and plasma, liver, spleen, mesenteric adipose, and brain hemispheres were collected. Liver and spleen were weighed and normalized to body weight and PAE animals were determined to have decreased normalized liver weight, an effect that was predominantly driven by males as revealed by post-hoc analyses. Additionally, leukocytes were isolated from blood, spleen, and cortex/hippocampus, ipsilateral and contralateral to the lesion at 2 days post-MCAO. Specifically, we saw an increase in circulating neutrophils in PAE animals, predominantly in males. Additionally, we observed a lower CD4+:CD8+ ratio in circulation and lower levels of IL-17 in circulating Th17 cells in PAE animals. Correlational analyses revealed sex-specific relationships between immune cell populations and stroke outcomes of infarct size and neurological score. Together, these data suggest potential mechanisms by which PAE animals may suffer worse outcomes from secondary insults that occur in adulthood. Understanding the impact of PAE on immune response post-stroke will result in better, FASD-informed health management of adults.

Disclosures: M.R. Pinson: None. **S. Bake:** None. **A.M. Adams:** None. **S. Pandey:** None. **F. Sohrabji:** None. **R.C. Miranda:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.03

Topic: A.07. Developmental Disorders

Support: NIAAA R01-AA023181

Title: Thalamic and basal forebrain nuclei are vulnerable to permanent cell loss after neonatal ethanol exposure

Authors: *J. F. SMILEY[1], P. VADDI[2], C. BLEIWAS[1], S. CANALS-BAKER[1], E. C. DIAS[1], D. A. WILSON[1], M. SAITO[1];
[1]Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY; [2]Nathan Kline Inst., Orangeburg, NY

Abstract: It is well known that neonatal ethanol exposure causes a spike of neuronal apoptosis in the brain, but for most brain structures the extent of permanent neuron loss that persists into adulthood is unknown. We and others previously demonstrated that cholinergic cells in the basal forebrain are especially vulnerable to this treatment, with as much as 40% reduction of cell number. In the present study we evaluated total neuron number in the basal forebrain (medial septal nucleus and vertical diagonal band), and compared the severity of neuron loss to that of several forebrain and brainstem neuron populations. C57Bl mice were injected with ethanol at postnatal day 7 (P7), and total Nissl-stained neuron numbers were evaluated by stereological methods at early adulthood (P70). Cell number estimates showed that ethanol caused especially severe reductions (20-40%) of total neuron number in both the basal forebrain region and the sub-nuclei of the anterior thalamic complex. In contrast, the cingulate cortex had a smaller reduction (10-20%) that was modestly more pronounced than that of total neocortex. Similarly, neuron numbers were not significantly reduced in cholinergic, dopamine and serotonin cells of the brainstem. The findings indicate that the basal forebrain and anterior thalamus are especially vulnerable to neonatal ethanol. Ongoing studies are investigating the mechanisms of ethanol vulnerability in these neuron populations. Supported by NIAAA R01-AA023181.

Disclosures: J.F. Smiley: None. **P. Vaddi:** None. **C. Bleiwas:** None. **S. Canals-Baker:** None. **E.C. Dias:** None. **D.A. Wilson:** None. **M. Saito:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.04

Topic: A.07. Developmental Disorders

Support: NINDS (R01NS100738 to J.T.)
LouLou Foundation (to J.T.)

Eunice Kennedy Shriver National Institute of Child Health and Human Development (U54HD083092, P50HD103555)
In Vivo Neurophysiology Core of Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital
Chao Family Foundation
Cockrell Family Foundation

Title: Cdkl5 deficiency augments inhibitory input into the dentate gyrus that can be reversed by deep brain stimulation

Authors: *Q. WANG[1,2], S. HAO[1,2], B. TANG[1,2], Z. WU[1,2], J. TANG[1,2];
[1]Jan & Dan Duncan Neurolog. Res. Inst., Houston, TX; [2]Dept. of Pediatrics, Baylor Col. of Med., Houston, TX

Abstract: CDKL5 deficiency disorder (CDD) is a neurodevelopmental disease caused by mutation of the X-linked gene cyclin-dependent kinase-like 5 (*CDKL5*). CDD is characterized by severe intellectual disability, gross motor impairment, early-onset epilepsy, and autistic features. Mouse models of CDD recapitulate the cognitive deficits and other characteristics of this condition. Although loss of CDKL5 affects a number of molecular pathways, very little has been discovered about the physiological effects of these changes on the neural circuitry. We therefore assessed the hippocampus-dependent behavior, synaptic plasticity, and local circuit activity in a unique CDKL5 knockout mouse model with exon 6 deletion (B6.129(FVB)-*Cdkl5*$^{tm1.1Joez}$/J). We found that CDKL5 haploinsufficiency in both male *Cdkl5*$^{-/y}$ and female *Cdkl5*$^{+/-}$ mice impair hippocampus-dependent learning and memory in multiple tasks including fear conditioning, passive avoidance, and Morris water maze. *In vivo*, loss of CDKL5 reduces long-term potentiation of the perforant path to the dentate gyrus and augments feedforward inhibition in this pathway. *Ex vivo*, *Cdkl5*$^{-/y}$ mice have decreased excitatory, but increased inhibitory synaptic transmission in dentate granule cells, confirming that excitatory/inhibitory input into the dentate gyrus is skewed toward inhibition after CDKL5 ablation. Furthermore, injecting the GABAergic antagonist gabazine into the dentate improved contextual fear memory in *Cdkl5*$^{-/y}$ mice. Finally, chronic forniceal deep brain stimulation rescued hippocampal memory deficits, restored synaptic plasticity, and relieved feedforward inhibition in *Cdkl5*$^{+/-}$ mice. Together, our findings suggest that CDKL5 is important for maintaining proper dentate excitatory/inhibitory balance for hippocampal memory and forniceal DBS as the possible translational intervention for treating CDD.

Disclosures: Q. Wang: None. **S. Hao:** None. **B. Tang:** None. **Z. Wu:** None. **J. Tang:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.05

Topic: A.07. Developmental Disorders

Support: R01 AA025967
R21 AA023051
T32-AA014127
P50 AA022534
R01 AA 019884

Title: Prenatal alcohol exposure enhances Toll like receptor (TLR4)-4 and NLRP3 inflammasome actions and paradoxically prolongs nerve injury-induced pathological pain following morphine treatment

Authors: *S. NOOR[1], M. S. SUN[1], *A. A. PASMAY[1], A. K. F. OROPEZA[1], C. D. RUFFANER-HANSON[1], M. V. V. NYSUS[2], D. C. JIMENEZ[1], D. D. SAVAGE[1], C. F. VALENZUELA[1], E. D. MILLIGAN[1];
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Abstract: Our prior work demonstrates that adult prenatal alcohol exposed (PAE) male rats with minimal nerve injury develop allodynia (pathological light touch sensitivity) in concert with aberrant peripheral and spinal glial and immune activation. Nerve-injured PAE rats display heightened proinflammatory cytokine production (IL-1β and TNF-α) typically produced following activation of the innate immune receptor, TLR4, and intracellular protein complex, Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes. This suggests that PAE-related adaptations prime peripheral macrophages and central nervous system (CNS) glia to over-respond to subtle challenges through exaggerated TLR4 and NLRP3 inflammasome reactivity. Interestingly, morphine, a standard opioid pain therapeutic, binds the μ-opioid receptor and glial TLR4 leading to NLRP3 inflammasome activation. Therefore, we hypothesized that PAE primes immune cell reactivity through augmented TLR4 activation and downstream NLRP3-mediated proinflammatory cytokine release, that is exacerbated by exposure to morphine treatment. We predicted morphine paradoxically prolongs allodynia following adult-onset peripheral nerve injury in PAE offspring through aberrant NLRP3 activation. Results confirm that PAE is a risk factor for developing allodynia in adult female mice with minor injury. Following minor nerve injury, morphine treatment (10 mg/kg, 5 subsequent days) significantly prolongs allodynia in PAE mice, while Sac control mice spontaneously resolve sooner. Moreover, selectively blocking NLRP3 inflammasome activity via MCC950 (i.v. 10 mg/kg) treatment reversed the morphine-prolongation of allodynia observed in PAE mice. *In vitro* studies were designed to examine the TLR4-NLRP3-dependent enhanced reactivity of PAE immune cells to typical immune stimuli with/without morphine treatment. Data show morphine further enhances NLRP3 inflammasome activity, as measured by flow cytometric detection of intracellular levels of active Caspase-1 and IL-1β release (by ELISA) from PAE leukocytes. *In vitro* culture data further confirmed MCC950 actions on inhibiting IL-1β release specifically, but not TNFα. Together, these data provide evidence that PAE exaggerates morphine-induced

immune activation via the TLR4-NLRP3 pathway and may be predictive of adverse responses to opioids intended for pain therapeutics in individuals with FASD.

Disclosures: S. Noor: None. **M.S. Sun:** None. **A.A. Pasmay:** None. **A.K.F. Oropeza:** None. **C.D. Ruffaner-Hanson:** None. **M.V.V. Nysus:** None. **D.C. Jimenez:** None. **D.D. Savage:** None. **C.F. Valenzuela:** None. **E.D. Milligan:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.06

Topic: A.07. Developmental Disorders

Support: NIH Grant F31 AA027694
NIH Grant R01AA023410
NIH Grant R01 AA027754

Title: A ROLE FOR CALCIUM SIGNALING IN THE ETHANOL-INDUCED ABERRANT TANGENTIAL MIGRATION OF PRIMORDIAL CORTICAL GABAERGIC INTERNEURONS

Authors: *S. M. LEE, P. W. L. YEH, H. H. YEH;
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Abstract: Alcohol consumption during pregnancy places the unborn child at risk for fetal alcohol spectrum disorders (FASD). While the diagnosis and management of FASD takes place postnatally, the underlying pathoetiology remains incompletely understood but is arguably embryonic. In mice, prenatal exposure to ethanol escalates tangential migration of cortex-bound primordial GABAergic interneurons, and this has been postulated to affect cortical circuit function and contribute to long-term excitatory/inhibitory imbalance. Previously, we found that ethanol potentiates $GABA_A$ receptor-mediated depolarization in tangentially migrating GABAergic interneurons. Here we investigated the downstream signaling mechanisms of ethanol-enhanced GABA depolarization underlying aberrant tangential migration.
First, we asked whether ethanol exposure potentiates calcium influx via L-type calcium channels (LTCC) in migrating GABAergic interneurons. Immunohistochemical staining confirmed that tangentially migrating Nkx2.1Cre/Ai14 GABAergic interneurons express Cav1.2 at embryonic day (E) 16.5, the canonical neuronal isoform of the LTCC. Exposure to liquid diet containing 5% ethanol from E13.5-16.5 did not change the expression levels of LTCC in the embryonic brain. In addition, whole-cell patch clamp electrophysiology revealed the presence of high-voltage activated nifedipine-sensitive LTCC in E16.5 Nkx2.1Cre/Ai14 embryos. This calcium influx

mediated by LTCC was potentiated following acute exposure to 18mM ethanol (80mg/dl or 0.08%), equivalent to 5% binge ethanol exposure paradigm *in vivo*.
Second, we assessed if LTCC is involved in ethanol-induced aberrant tangential migration of GABAergic interneurons. To this end, we treated organotypic slice cultures with control, nifedipine, ethanol, and nifedipine + ethanol-containing medium. Co-treatment of ethanol and nifedipine prevented the effect of ethanol on migration. We found that maternal treatment from E13.6 to E16.5 with liquid diet containing 5% ethanol along with nifedipine (0.15mg/kg body weight) also normalized tangential migration *in vivo*.
The present study provides a mechanistic link implicating calcium as an important intermediary in the ethanol-induced aberrant migration of immature GABAergic cortical interneurons reported in our mouse model of FASD.
Supported by PHS Grants F31 AA027694, R01AA023410, and R01 AA027754.

Disclosures: S.M. Lee: None. **P.W.L. Yeh:** None. **H.H. Yeh:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.07

Topic: A.07. Developmental Disorders

Support: NIH, DC-IDDRC 1U54HD090257-01
NIH, NIAAA F31AA027693

Title: Activation of the anterior cingulate cortex ameliorates anxiety in a preclinical model of fetal alcohol spectrum disorders

Authors: *H. M. HWANG, K. HASHIMOTO-TORII;
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Abstract: Prenatal alcohol exposure (PAE) results in a broad range of neurobehavioral issues in patients with fetal alcohol spectrum disorders (FASD). One prominent lifelong comorbidity in these patients is anxiety. Both preclinical and clinical studies suggest that disrupted hypothalamic-pituitary-adrenal (HPA) axis is a reason for increased risk of anxiety disorders in FASD. However, changes in brain circuitry or molecular pathways, other than the HPA axis, have not yet been elucidated fully in FASD. The anterior cingulate cortex (ACC) is one of the brain regions associated with anxiety. Studies show malfunctioning of the ACC is associated with hyperactivity of the amygdala - the hub for fear processing - in patients with anxiety disorders, and stimulation of the ACC decreases neuronal activity in the amygdala. In this study, we evaluated whether ACC and amygdala connectivity plays a role in anxiety-like phenotype

due to PAE. Pregnant CD1 mice were exposed to alcohol (4g/kg body weight) at gestational day 16 and 17, during the peak of corticogenesis, and offsprings were assessed for behavioral changes at postnatal day 30. Behavioral analysis revealed a typical anxiety-like phenotype in PAE group: significant reductions in self-grooming time and open arm time in the elevated plus maze (EPM) compared to control group. We also found that stress hormone (corticosterone or CORT) concentration is significantly high in PAE group in both resting state and after EPM test, and their level significantly correlated with open arm time. C-Fos, immediate early gene marker, immunohistochemistry identified significantly decreased activity in the ACC but increased activity in the basolateral amygdala (BLA) in PAE group after EPM test. Furthermore, double-immunofluorescence of c-Fos with inhibitory (PV) or excitatory (VGLUT2) markers showed a significant loss of excitatory neuronal activations in the ACC. To confirm if the reduction in ACC activity in PAE group is responsible for anxiety-phenotype, we chemogenetically stimulated excitatory neurons employing the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) method. Clozapine N-oxide (CNO) administration to AAV5-CaMKIIa-hM3D(Gq)-mCherry virus injected PAE mice showed a significant improvement in open arm time in the EPM test accompanied by a significant increase of c-Fos level in the ACC but a decrease in the BLA. In summary, our data suggest that a decreased activity of excitatory neurons in the ACC that regulate BLA activity due to PAE underlies anxiety-like behavior and demonstrate that stimulating the ACC can be a potential regimen to alleviate anxiety symptoms in FASD patients.

Disclosures: H.M. Hwang: None. **K. Hashimoto-Torii:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.08

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01NS106285

Title: Forebrain-specific knockout of Cited2 leads to transcriptome disruptions in intermediate progenitor cells and results in disrupted neocortical developmental behavioral alterations

Authors: *N. WAGNER, J. L. MACDONALD;
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Abstract: The mammalian neocortex develops from a single layer of neuroepithelial cells to form a six-layer heterogeneous mosaic of differentiated neurons and glial cells through a complex choreography of temporally and spatially restricted transcription factors and epigenetic

regulators. One recently identified epigenetic regulator that is essential for the precise development of the neocortex is CITED2 (CBP/p300 Interacting Transactivator with ED-rich termini). *Cited2* is highly expressed by intermediate progenitor cells (IPCs) in the subventricular zone during the generation of the superficial layers of the neocortex at embryonic day 15.5 (E15.5). Previous work showed that mice with a forebrain-specific conditional knockout of *Cited2* (cKO) exhibit reduced proliferation of IPCs embryonically through previously unresolved mechanisms. This leads to reduced thickness of the superficial layers, reduced corpus callosum volume, and disruptions in balanced neocortical arealization. We have found that these morphological abnormalities are sufficient to generate behavioral alterations that are associated with neurodevelopmental disorders, including decreased ultrasonic vocalizations as neonates, and an increase in rearing behavior and lack of habituation following repeated acoustic startle as adults. Excitingly, our data suggest that *in utero* folic acid supplementation is sufficient to ameliorate neocortical morphological abnormalities in the *Cited2* cKO, though the effect of folic acid supplementation on cKO behavior remains unknown. Ongoing work is aimed at uncovering the molecular mechanisms by which CITED2 functions in IPCs to regulate neocortical development and behavior. We compare the transcriptomes of purified IPCs from the developing cortex of E15.5 *Cited2* wild-type and cKO littermates to identify genes that are regulated by CITED2 in the developing neocortex. Taken together, these results identify the *Cited2* cKO as a novel system to test how *in utero* environmental exposures lead to altered neocortical development and life-long alterations in behavior.

Disclosures: N. Wagner: None. **J.L. MacDonald:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.09

Topic: A.07. Developmental Disorders

Support: Grant n° 31003A-175778

Title: New in vivo AAV strategy to treat SLC6A8 deficiency

Authors: *G. FERNANDES-PIRES[1], L. DURAN-TRIO[2], M. LANZILLO[2], L. TENENBAUM[3], O. BRAISSANT[1];
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Abstract: BACKGROUND: Creatine (Cr) is synthetized by the enzymes AGAT and GAMT, and specifically taken up by the transporter SLC6A8. Deficit in its endogenous synthesis or transport leads to Cerebral Creatine Deficiency Syndromes (CCDS). AGAT, GAMT and SLC6A8 deficiencies are inborn error of metabolism (IEM) characterized by brain Cr deficiency causing neurodevelopmental delays and intellectual disability. While AGAT and GAMT deficiencies are improved with Cr treatment, SLC6A8 deficiency cannot. We develop adeno-associated virus (AAV) gene therapy in our SLC6A8$^{Y389C/y}$ knock-in rat model as proof of concept for SLC6A8 deficiency treatment. We present here AAV9 CNS transduction of both WT and *Slc6a8*$^{Y389C/y}$ rat males with mCherry fluorescent protein and functional Slc6a8 transporter.
METHODS: WT and KI males were injected at postnatal day 11 intracisternally with 10µl of ss-AAV9-2YF-mCherry or ssAAV9-2YF-CMV-SLC6A8-Flag suspensions (10^{12} vg/ml). Animals were sacrificed at 5 and 16 weeks post-injection to assess AAV9 transduction efficacy and cellular tropism. Starting from 5 weeks post-injection, a subgroup of ssAAV9-2YF-CMV-SLC6A8-Flag-injected KI males were exposed to daily Cr treatment (2g/kg*day).
RESULTS: We observed a widespread mCherry transduction in most CNS regions (cortex, hippocampus, medulla oblongata, cerebellum, spinal cord). As compared to non-injected KI males, ssAAV9-2YF-CMV-SLC6A8-Flag-injected KI males showed partial restablishment of body weight gain and body-mass index (BMI).
DISCUSSION: Our strategy appears as promising to treat SLC6A8 deficiency, with encouraging brain Cr increase as well as body weight and BMI corrections. While still effort-demanding in terms of improvement, this approach may become available in near future to treat the so far untreatable SLC6A8 deficiency.

Disclosures: G. Fernandes-Pires: None. **L. Duran-Trio:** None. **M. Lanzillo:** None. **L. Tenenbaum:** None. **O. Braissant:** None.

Poster

P061. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P061.10

Topic: A.07. Developmental Disorders

Support: NIH R01 NS097237
NIH R01 AG045036
NIH R01 NS085152
NIH DC017390
NIH R21 NS096402
NFRP W81XWH-16-1-0220
The Kanter Family

Title: Neuronal and molecular mechanisms of Nf1-dependent metabolic regulation

Authors: *V. BOTERO[1], B. STANHOPE[2], E. BROWN[2], E. GRENCI[1], T. BOTO[1], S. PARK[1], L. KING[1], K. MURPHY[1], K. J. COLODNER[3], J. A. WALKER[4,5], A. C. KEENE[2], W. W. JA[1], S. M. TOMCHIK[1];
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Abstract: Neurofibromatosis type 1 (NF-1) is an autosomal dominant genetic disorder predisposing patients to a range of complications, including benign tumor formations in the nervous system, altered cellular function, short stature, bone abnormalities, and increased rates of cognitive and developmental disorders. NF-1 is caused by mutations in the *NF1* gene, which encodes neurofibromin (Nf1), a large protein that functions as a negative regulator of Ras signaling and mediates pleiotropic organismal and cellular functions. Emerging evidence suggest that Nf1 regulates metabolism: NF-1 patients show a reduced body mass index, alterations in metabolites, lower incidences of diabetes, and increased resting energy expenditure. These changes in metabolism may contribute to complications and symptoms associated with NF-1. The extent and mechanisms by which Nf1 affects metabolism and energy expenditure are not well understood. Using the *Drosophila melanogaster NF1* ortholog, we show that Nf1 regulates metabolic homeostasis via neuronal mechanisms. *Drosophila* Nf1 is ~60% identical to the human protein and similarly mediates Ras signaling. Our data show that the loss of Nf1 increases metabolic rate via a Ras-GAP-related domain, increases metabolic rate, feeding rate, starvation susceptibility, and alters lipid stores and turnover kinetics. These metabolic alterations are independent of locomotion and grooming activity and map to a restricted subset of neurons in the ventral nervous system. Acute activation of this neural circuit mimics the loss of Nf1 function by increasing metabolic rate when stimulated. These data indicate that Nf1 may regulate changes in neuronal metabolic control, suggest that cellular and systemic metabolic alterations may be a pathophysiological mechanism of NF-1, and provide a platform for investigating the cellular role of neurofibromin in metabolic homeostasis.

Disclosures: V. Botero: None. **B. Stanhope:** None. **E. Brown:** None. **E. Grenci:** None. **T. Boto:** None. **S. Park:** None. **L. King:** None. **K. Murphy:** None. **K.J. Colodner:** None. **J.A. Walker:** None. **A.C. Keene:** None. **W.W. Ja:** None. **S.M. Tomchik:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.01

Topic: B.03. G-Protein Coupled Receptors

Support: R33DA041876

Title: Conditional spinophilin knockout mice as a novel model for detailing striatal MSN-specific mechanism underlying rodent repetitive behavior

Authors: *C. W. MORRIS, D. S. WATKINS, A. J. BAUCUM, II;
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Abstract: Dysfunction of metabotropic glutamate receptor 5 (mGluR5) activity in striatal medium spiny neurons (MSNs) is associated with numerous psychiatric disorders in humans and repetitive grooming in rodents, an evolutionarily conserved motor repertoire composed of a complex pattern of motor actions. Therefore, understanding striatal-specific signaling mechanisms by which mGluR5 causes repetitive grooming can increase our knowledge of how motor programs become pathological. Spinophilin, the most abundant protein-phosphatase 1 (PP1) targeting protein in the striatal post-synaptic density (PSD), prevents activity-dependent endocytosis of mGluR5 in heterologous cells and primary cortical neuron cultures. However, the extent to which spinophilin functions in striatal MSNs to mediate mGluR5-driven grooming dysfunction is unclear. To address this, we introduce a conditional spinophilin knockout (KO) mouse model to detail MSN-specific mechanisms by which spinophilin regulates mGluR5 function using behavioral and biochemical analyses. Behaviorally, we detail spinophilin's MSN-specific roles in mediating acute and chronic forms of mGluR5-driven repetitive grooming. Biochemically, we determine that spinophilin's protein interaction with mGluR5 in rodent striatum positively correlates with basal and pathological grooming behavior. Lastly, we introduce the use of adeno-associated viruses (AAVs) to deplete or replete spinophilin expression in adult striatal MSNs to draw a causal relationship between spinophilin and mGluR5 function. Overall, our results suggest spinophilin is critical for mGluR5-dependent striatal plasticity underlying grooming pathology in rodents. Moreover, we conclude that spinophilin, and/or MSN-specific spinophilin interacting proteins, may serve as future therapeutic targets to fractionate pathologically grouped actions in psychiatric disorders, such as obsessive-compulsive spectrum disorders (OCSDs) and autism spectrum disorder (ASD).

Disclosures: C.W. Morris: None. **D.S. Watkins:** None. **A.J. Baucum:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.02

Topic: B.03. G-Protein Coupled Receptors

Support: MH119673
NS031373

MH062646
MH073676
MH065215

Title: Selective $mGlu_1$ potentiation enhances cortical inhibition to rescue schizophrenia-like cognitive and social deficits induced by phencyclidine (PCP)

Authors: *D. J. LUESSEN[1,3], I. GALLINGER[2,3], J. T. MAKSYMETZ[4], P. CONN[1,3];
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Abstract: Accumulating evidence suggests that selective activators of the $mGlu_1$ subtype of metabotropic glutamate (mGlu) receptor may provide a promising novel therapeutic approach for the treatment of schizophrenia. Genetic studies in humans reveal an association of the human gene encoding $mGlu_1$ (*GRM1*) and, specifically, loss of function single nucleotide polymorphisms in *GRM1* with schizophrenia, raising the possibility that $mGlu_1$ signaling is critical to the function of brain circuits underlying symptoms associated with this disorder. Multiple clinical and preclinical studies support the hypothesis that loss of GABAergic inhibitory transmission in the prefrontal cortex (PFC) and other forebrain regions may play a critical role in the pathophysiological changes underlying behavioral deficits in schizophrenia patients, including cognitive and social impairment. This is especially interesting in light of our recent studies demonstrating that activation of $mGlu_1$ increases inhibitory transmission in the PFC by selective excitation of somatostatin-expressing GABA interneurons. Here, we leverage recently developed positive allosteric modulators (PAMs) selective for $mGlu_1$ to examine whether $mGlu_1$ activation might reverse electrophysiological effects and behavioral deficits induced by subchronic treatment with phencyclidine (scPCP), an NMDA receptor antagonist commonly used to model cortical pathophysiology relevant to behavioral and cognitive deficits observed in schizophrenia patients. Using ex vivo whole-cell patch-clamp electrophysiology, we found that scPCP decreased the frequency of spontaneous inhibitory postsynaptic currents onto layer V pyramidal cells of the PFC. This cortical disinhibition was reversed by $mGlu_1$ activation, suggesting that $mGlu_1$ activation is a pertinent mechanism to restore inhibitory signaling in the PFC. Importantly, the $mGlu_1$ PAM VU6004909 effectively reversed scPCP-induced deficits in prepulse inhibition of acoustic startle, novel object recognition and social interaction in a three-chamber assay. In combination with our previous reports that $mGlu_1$ PAMs possess antipsychotic-like efficacy and reverse deficits in inhibitory transmission in the PFC, the current study provides strong evidence that $mGlu_1$ PAMs could serve as a novel approach that could reduce positive symptoms while also treating cognitive and social deficits in schizophrenia patients, providing an exciting improvement over current antipsychotics.

Disclosures: D.J. Luessen: None. **I. Gallinger:** None. **J.T. Maksymetz:** None. **P. Conn:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.03

Topic: B.03. G-Protein Coupled Receptors

Support: R01MH122545
R01MH119673

Title: Antipsychotic-like effects of a CB_2 PAM in mice

Authors: *D. J. FOSTER, T. ISHMAM, J. KALBFLEISCH, C. LINDSLEY, P. J. CONN; Warren Ctr. For Neurosci. Drug Discovery, Nashville, TN

Abstract: Currently approved drugs for treating schizophrenia all target D_2 dopamine receptors. While these drugs show efficacy in the clinic, this efficacy often does not translate across different symptom domains, and a subset of patients are treatment-resistant. Novel therapeutic strategies that diverge from targeting the D_2 receptor are desperately needed to provide a more diverse arsenal of therapeutic options. We and others have previously demonstrated that positive allosteric modulators (PAMs) targeting the M_4 muscarinic receptor can induce robust antipsychotic-like effects in numerous rodent models. We previously reported a surprising role for CB_2 cannabinoid receptors in that these receptors are required for M_4 PAM-mediated effects on regulating dopamine signaling and mediating antipsychotic-like efficacy. These results raised the possibility that targeting the CB_2 receptor directly could be a novel strategy for achieving antipsychotic-like efficacy. Here we report that administration of a CB_2 PAM can reverse amphetamine-induced deficits in sensorimotor gating and that these effects are blocked by the CB_2 antagonist AM630. We go on to show that administration of CB_2 PAM reduces the hyperlocomotion induced by either amphetamine or the NMDA receptor antagonist MK-801. These results indicate that directly targeting the CB_2 receptor is sufficient to mediate antipsychotic-like efficacy and provide additional evidence that the CB_2 receptor could be an exciting novel target for treating schizophrenia.

Disclosures: D.J. Foster: None. **T. Ishmam:** None. **J. Kalbfleisch:** None. **C. Lindsley:** None. **P.J. Conn:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.04

Topic: B.03. G-Protein Coupled Receptors

Support: National Science Centre, Poland, grant 2016/20/S/NZ7/00424

Title: Alterations of hippocampal free intracellular zinc and total serum zinc levels in GPR39 knockout mice subjected to pentylenetetrazole kindling model of epilepsy and treatment with TC-G 1008

Authors: *U. DOBOSZEWSKA[1,2], J. SAWICKI[3], A. RAFALO-ULINSKA[4], K. SOCALA[2], M. PIEROG[2], D. NIEOCZYM[2], B. SZEWCZYK[4], M. MACKOWIAK[4], I. SOWA[3], P. WLAZ[2]; [1]Jagiellonian Univ. Med. Col., Krakow, Poland; [2]Animal Physiol. and Pharmacol., Maria Curie Sklodowska Univ., Lublin, Poland; [3]Analytical Chem., Med. Univ. of Lublin, Lublin, Poland; [4]Maj Inst. of Pharmacol., Polish Acad. of Sci., Krakow, Poland

Abstract: Introduction: The G-protein coupled receptor 39 (GPR39) is activated by zinc ions and was suggested as a novel drug target for treating epileptic seizures. TC-G 1008 is a highly specific agonist of GPR39. We found that TC-G 1008 facilitated the development of pentylenetetrazole (PTZ) kindling. Using GPR39 knockout (KO) mouse line we verified that GPR39 is a target for TC-G 1008 in regard to PTZ-induced epileptogenesis. Our results argue against the hypothesis that activation of GPR39 alleviates seizures. Free zinc is responsible for signaling, both extracellularly (for example at GPR39) and intracellularly. Free intracellular zinc ($[Zn^{2+}]_I$) may be associated with epileptogenesis. We therefore examined this pool of zinc in GPR39 KO in the PTZ model of epilepsy. **Methods:** GRP39 KO mouse model was generated by the Mouse Genome Engineering Facility (crisprmice.eu). The model was generated in mixed genetic background (C57BL6/Tar x CBA/Tar), a deletion of 44 bp causing p.Lys38fs*57X frameshift mutation was introduced. Male GPR39 KO and WT mice were subjected to PTZ-kindling (approval number 72/2019 of the Local Ethical Committee in Lublin). GPR39 KO and WT mice received vehicle or TC-G 1008 (10 mg/kg, i.p.) 30 min before each dose of PTZ (25 mg/kg, i.p.). 24 h after the last dose of PTZ, tissue was harvested. Total serum zinc concentration was measured using Inductively Coupled Plasma Optical Emission Spectrometry. $[Zn^{2+}]_I$ was measured in hippocampal sections using membrane-permeable fluorescent probe Zinpyr-1. The sections were double stained with DAPI. Additional sections were treated with membrane-permeable zinc chelator, TPEN, before staining with Zinpyr-1 and DAPI. The sections were imaged using Leica DM6000 B microscope. Data were analyzed using Image J and Graph Pad Prism v. 5.03, by the Two-way ANOVA and a Bonferroni multiple comparison test. **Results:** Serum zinc concentration was decreased in GPR39 KO mice subjected to PTZ-kindling, compared to WT mice subjected to PTZ kindling. Chronic administration of TC-G 1008 did not affect serum zinc concentration in either GPR39 KO or WT mice subjected to this procedure. $[Zn^{2+}]_I$ did not difer between GPR39 KO and WT in this model. Moreover, chronic treatment with TC-G 1008 increased $[Zn^{2+}]_I$ in the CA3, CA1 and DG regions of the hippocampus of WT mice, but not in GPR39 KO mice. **Conclusion:** Increased $[Zn^{2+}]_I$ in WT mice chronically treated with TC-G 1008 may be associated with increased maximal seizure score in these mice (our previous data), thus suggesting that $[Zn^{2+}]_I$ may participate in aggravation of epileptogenesis following activation of GPR39. Serum zinc is not a marker for TC-G 1008 administration.

Disclosures: U. Doboszewska: None. **J. Sawicki:** None. **A. Rafalo-Ulinska:** None. **K. Socala:** None. **M. Pierog:** None. **D. Nieoczym:** None. **B. Szewczyk:** None. **M. Mackowiak:** None. **I. Sowa:** None. **P. Wlaz:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.05

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant AA025403
NIH Grant AA020889

Title: Metabotropic receptor 2 deletion effects on behavioral flexibility in mice

Authors: *M. LITTLEPAGE-SAUNDERS[1], M. J. HOCHSTEIN[1], K. AMELGA[1], G. E. HOMANICS[2], K. A. JOHNSON[3];
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Abstract: Substance use disorders (SUD) are chronic brain disorders that affect more than 19 million adults in the United States (SAMHSA, 2018). In order to develop more effective treatments for SUD, it is key to understand the brain mechanisms and behavioral adaptations that contribute to drug misuse including high levels of drug consumption. Preclinical studies have linked loss of metabotropic glutamate receptor 2 (mGlu2) function with increased vulnerability to heavy consumption of drugs including alcohol, heroin, and cocaine. However, the mechanisms by which loss of mGlu2 function enhance drug consumption are not fully understood. Because mGlu2 is a major regulator of glutamatergic transmission in the basal ganglia, and basal ganglia glutamatergic transmission plays important roles in addiction-related behavioral processes such as learning, action control, and behavioral flexibility, it is possible that impaired mGlu2 function leads to changes in these behaviors that might facilitate heavy or inflexible drug use. To test this, we evaluated adult male and female mice with a conditional deletion of mGlu2 from glutamatergic telencephalic neurons including cortical neurons (mGlu2 flox;Emx1-IRES-Cre mice) in operant behavior tasks that assess goal-directed vs. habitual action control. We trained mice to lever press for palatable food pellets on a random ratio reinforcement schedule, then performed devaluation and contingency degradation experiments to assess the effects of mGlu2 deletion on goal-directed and habitual action control. Compared with control mice, male mice lacking mGlu2 showed impaired contingency degradation. However, male mice lacking mGlu2 demonstrated normal devaluation after reinforcer prefeeding. This finding suggests that in male mice, mGlu2 deletion specifically impairs the ability to update behavior in response to a change in action-outcome contingency. Ongoing experiments will evaluate potential sex differences in mGlu2 regulation of habitual behaviors. Future studies will assess how circuit-specific impairments in mGlu2 function, particularly in corticostriatal circuits, contribute to inflexible behaviors including habits.

Disclosures: M. Littlepage-Saunders: None. **M.J. Hochstein:** None. **K. Amelga:** None. **G.E. Homanics:** None. **K.A. Johnson:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.06

Topic: B.03. G-Protein Coupled Receptors

Support: NIDA R01-DA042156

Title: Prolonged amphetamine exposures increase the endogenous human dopamine receptors 2 at the cellular membrane in cells lacking the dopamine transporter

Authors: V. NAWARATNE, ***S. P. MCLAUGHLIN***, F. P. MAYER, Z. GICHI, A. MASTRIANO, L. CARVELLI;
Florida Atlantic Univ., Jupiter, FL

Abstract:

Disclosures: V. Nawaratne: None. **S.P. McLaughlin*:** None. **F.P. Mayer:** None. **Z. Gichi:** None. **A. Mastriano:** None. **L. Carvelli:** None.

Poster

P090. Metabotropic Receptors: Behavioral Effects

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P090.07

Topic: B.03. G-Protein Coupled Receptors

Support: NIMH-IRP-1ZIAMH002386

Title: Constitutive and acute effects of PACAP gene knockout on behavior, physiology, and the transcriptome

Authors: *D. BAKALAR[1], S. C. SWEAT[1], G. L. DROSSEL[1], S. Z. JIANG[1], B. S. SAMAL[1], N. STROTH[1], W. XU[1], L. ZHANG[2], H. ZHANG[1], L. E. EIDEN[1];
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Abstract: The role of neuropeptides in brain function has been investigated both via the use of pharmacological agonists and antagonists to provoke or inhibit outputs and using mice deficient in the peptide or its receptor(s) (knockout mice). Functional deficiencies in adult neuropeptide-deficient mice could be due to genetic or non-genetic immediate effects at the time of experimentation, or to effects occurring during development. These differences in turn could reflect developmental or neuromodulatory roles for a given neuropeptide. To dissociate the acute versus constitutive transcriptomic, physiological, and behavioral effects of PACAP deficiency, we examined the *Adcyap1* KO mouse and the corresponding *Adcyap1r1* KO mouse. Using microarray, we demonstrate that in the constitutively (from conception) *Adcyap1* (PACAP) knockout mouse there is a cohort of genes (constitutively PACAP-Regulated Genes, or cPRGs) whose basal expression is altered in PACAP-deficient mice as compared to wildtype controls in multiple brain regions, and additional genes (acutely PACAP-Regulated Genes, or aPRGs) whose basal expression is the same in wild-type and knockout mice, but whose alteration in response to a physiological challenge is impaired in the absence of PACAP expression. The identified genes are confirmed with qRT-PCR. We identify a behavioral phenotype (repetitive jumping) which is dramatic in constitutive *Adcyap1* KO mice, but is not phenocopied by either the PACAP receptor PAC1 KO or by post-natal knockout of *Adcyap1*. This indicates a developmental rather than an acute role of PACAP that may be reflected in the expression of cPRGs, such as *Pttg1* and *Xaf1*, as no changes in these cPRGs are detected by qRT-PCR in post-natal *Adcyap1* knockout tissue or by microarray in PAC1 KO tissue. On the other hand, blunted responses to restraint stress (hypophagia and corticosterone elevation) seen in PACAP-deficient mice are phenocopied in PAC1 deficient mice, suggesting a 'real-time' (neuromodulatory) role for PACAP-PAC1 signaling. Distinguishing constitutive and acute effects of neuropeptide deficiency reveals the changing functions of neuropeptides through the lifespan.

Disclosures: D. Bakalar: None. **S.C. Sweat:** None. **G.L. Drossel:** None. **S.Z. Jiang:** None. **B.S. Samal:** None. **N. Stroth:** None. **W. Xu:** None. **L. Zhang:** None. **H. Zhang:** None. **L.E. Eiden:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.01

Topic: B.10. Epilepsy

Support: NIH NINDS F32, NS 114322
NIH NINDS K23, NS 114178

Title: Patient-specific structural connections are predictive of seizure propagation pathways

Authors: *D. N. ANDERSON[1], E. H. SMITH[1], J. E. LEWIS[1], C. M. CHARLEBOIS[2], B. J. PHILIP[1], B. L. JOHNSON[3], A. Y. PETERS[3], B. J. NEWMAN[3], A. M. ARAIN[3], T. S. DAVIS[4], K. S. WILCOX[5], J. D. ROLSTON[1];
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Abstract: *Rationale.* Patients experiencing drug-resistant seizures may undergo intracranial monitoring to localize their seizure onset zone; however, high patient-to-patient variation in the seizure onset zone may complicate diagnosis and may explain the mixed success in therapies across epilepsy patients. Evaluation of patient-specific networks may help identify important pathways in each patient's seizure propagation network. The purpose of this study was to correlate seizure propagation to patient-specific structural imaging with the long-term goal of improving targeting of the seizure network through surgical intervention. *Methods.* We analyzed a cohort of patients (n=7) undergoing invasive stereo-electroencephalography (SEEG) at the University of Utah. We acquired pre-operative MRI, diffusion-weighted imaging (DWI), and post-operative CT. We localized and registered the SEEG electrodes in the post-operative CT to the structural MRI and DWI. Using the clinically defined seizure onset zone (SOZ) as the seed location, we calculated probabilistic tractography and quantified the likelihood of axonal connections and average path length from the SOZ to each SEEG contact, as defined by a 1 cm radius around each contact's centroid. Using wavelet spectrogram analysis, we determined the seizure onset time based on when the power in the dominant frequency band surpassed the median power for each channel. We correlated the probability of axonal connections and the average connection length to the time of seizure onset for each SEEG contact using Pearson's correlation. *Results.* A total of 588 SEEG contacts were implanted across 7 patients, who experienced 44 clinical seizures across 10 clinically defined SOZs. We found that connection probability and path length were statistically significant predictors of seizure onset times per channel (*t*-test, $p < 0.05$) for 18/44 seizures and 23/44 seizures, respectively. Importantly, patients with significant structure and function correlations for the majority of their seizures had better clinical outcomes (Engel score I or II) compared to patients with poor structure and function correlations (Kruskal-Wallis, Tukey-Kramer post-hoc, $p < 0.05$). *Conclusions.* These results demonstrate that increased connections and shorter path lengths from the SOZ may predict seizure propagation pathways. Based on outcome scores, a better structure and function correlation may correlate to more precise SOZ localization. Isolating seizure propagation pathways through structural connectivity has the potential to improve targeting of surgical interventions, and thus improve clinical outcomes.

Disclosures: D.N. Anderson: None. **E.H. Smith:** None. **J.E. Lewis:** None. **C.M. Charlebois:** None. **B.J. Philip:** None. **B.L. Johnson:** None. **A.Y. Peters:** None. **B.J. Newman:** None. **A.M. Arain:** None. **T.S. Davis:** None. **K.S. Wilcox:** None. **J.D. Rolston:** F. Consulting Fees (e.g., advisory boards); Medtronic, Inc., Corlieve Therapeutics.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.02

Topic: B.10. Epilepsy

Support: Tiny Blue Dot Foundation

Title: Differences in topography of slow wave activity between focal seizures with versus without impaired consciousness and sleep: a high-density EEG study

Authors: K. SHOGER[1], ***A. SELTE**[1], B. SEVAK[1], R. VERHAGEN[2], E. JUAN[3], G. FINDLAY[1], T. BUGNON[1], A. MENSEN[1], R. MAGANTI[1], G. TONONI[1], A. STRUCK[1], M. BOLY[1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Vrije Univ., Amsterdam, Netherlands; [3]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Rationale: Previous intracranial EEG (iEEG) studies suggested that impaired consciousness during focal seizures is linked to increases in slow-wave activity (SWA). Here we used high-density EEG (HDEEG) to investigate topographical differences between focal seizures with preserved vs impaired consciousness (FA vs FIA), wake and non-rapid eye movement (NREM) sleep.
Methods: Ictal HDEEG recordings were performed on focal epilepsy patients at the University of Wisconsin-Madison Epilepsy Monitoring Unit during two seizure types: FIA (n = 19 from 7 patients, mean age 43 ± SD 11, 4 females; focus location in bilateral temporal: n =2; R temporal: n = 4; and R occipital: n = 1) and FA (n = 7 from 3 patients, mean age 38 ± SD 9, 1 female; focus locations in R temporal: n = 2; and R occipital: n = 1). HDEEG data were filtered between 1-25 Hz, with visual rejection of noisy epochs and channels. Seizure data was concatenated with sleep (N2 and N3 stages, matching 18/19 FIA and 5/7 FA) and wake data before Independent Component Analysis. Topographies of EEG power in the SWA (delta, 1-4 Hz), theta (4-8 Hz) and sigma (12-15 Hz) bands and delta/beta (15-25 Hz) ratio were compared between FA, FIA, wake, and N2-N3 sleep (both for absolute and spatially Z-scored values). Random effects analysis used Statistical Parametric Mapping software (threshold set at $p<0.05$ with correction for multiple comparisons at voxel or cluster levels).
Results: Compared to both FA and wake, FIA seizures were characterized by more widespread increases in SWA, theta and spindle power. However, there was a significant topographical difference: FIA seizures displayed more anterior frontal SWA while FA showed significantly more central SWA (Z-scored analysis). Interestingly, spindle power was increased both in frontotemporal and central channels during both FA and FIA compared to wake; but significantly less SWA and spindle power were present during focal seizures than during N2 and N3 sleep.
Conclusion: Altogether, these results suggest sleep-like activities even during seizures with preserved awareness (FA), with a pattern resembling light NREM sleep. FIA seizures showed more widespread sleep-like activities compared to both FA and wake, with a topography

resembling deeper NREM sleep. Interestingly, the involvement of medial prefrontal cortex in SWA and spindle activities were decreased overall during focal seizures compared to sleep, suggesting differences in the involvement of subcortical arousal systems.

Disclosures: K. Shoger: None. **A. Selte:** None. **B. Sevak:** None. **R. Verhagen:** None. **E. Juan:** None. **G. Findlay:** None. **T. Bugnon:** None. **A. Mensen:** None. **R. Maganti:** None. **G. Tononi:** None. **A. Struck:** None. **M. Boly:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.03

Topic:

Support: NIH NINDS K23NS112339

Title: Low frequency novel interictal EEG biomarker for localizing seizures and predicting outcomes

Authors: *B. N. LUNDSTROM, B. BRINKMANN, G. A. WORRELL;
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Abstract: Objective and Rationale: Localizing hyperexcitable brain tissue to treat focal seizures remains challenging. We want to identify the seizure onset zone (SOZ) from interictal EEG biomarkers. We hypothesize that a combination of interictal EEG biomarkers, including a novel low frequency marker, can predict mesial temporal involvement and can assist in prognosis related to surgical resections. **Methods and Results:** Interictal direct current wide bandwidth invasive EEG recordings from 83 patients implanted with 5,111 electrodes were retrospectively studied. Logistic regression was used to classify electrodes and patient outcomes. A feed-forward neural network was implemented to understand putative mechanisms. Interictal infraslow frequency EEG activity was decreased for SOZ electrodes while faster frequencies such as delta (2-4 Hz) and beta-gamma (20-50 Hz) activity were increased. These spectral changes comprised a novel interictal EEG biomarker that was significantly increased for mesial temporal SOZ electrodes compared to non-SOZ electrodes. Interictal EEG biomarkers correctly classified mesial temporal SOZ electrodes with a specificity of 87% and positive predictive value of 80%. These interictal EEG biomarkers also correctly classified patient outcomes after surgical resection with a specificity of 91% and positive predictive value of 87%. **Conclusions:** Interictal infraslow EEG activity is decreased near the SOZ while higher frequency power is increased, which may suggest distinct underlying physiologic mechanisms. Narrowband interictal EEG power bands provide information about the SOZ and can help predict mesial temporal involvement in seizure onset. Narrowband interictal EEG power bands may be less useful for

predictions related to non-mesial temporal electrodes. Together with interictal epileptiform discharges and high frequency oscillations, these interictal biomarkers may provide prognostic information prior to surgical resection. Computational modeling suggests changes in neural adaptation may be related to the observed low frequency power changes.

Disclosures: B.N. Lundstrom: 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.; Medtronic EPAS, NeuroPace RESPONSE, Neuroelectrics tDCS for Epilepsy. **B. Brinkmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience, Inc. **G.A. Worrell:** 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.; Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience, Inc, NeuroOne.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.04

Topic: B.10. Epilepsy

Support: Tiny Blue Dot Foundation

Title: Signatures of loss of consciousness during focal impaired awareness (FIA) and focal aware (FA) seizures.

Authors: *U. GORSKA[1], C. KOZMA[1], C. PAPANTONATOS[2], M. GROBBELAAR[1], E. JUAN[3,1], A. STRUCK[2], H. BLUMENFELD[4], G. TONONI[1], M. BOLY[1,2];
[1]Dept. of Psychiatry, [2]Dept. of Neurol., Univ. of Wisconsin-Madison, Madison, WI; [3]Univ. of Amsterdam, The Netherlands, Netherlands; [4]Yale Sch. of Med., New Haven, CT

Abstract: Loss of consciousness (LOC) occurring during epileptic seizures can cause life-threatening repercussions. While it was shown that cortical sleep-like activities accompany LOC during focal impaired awareness seizures (FIAS), this activity was not yet explicitly studied in focal aware seizures (FAS).
Here we quantified clinical signs of LOC based on videoEEG review and scoring of both behavioral responsiveness and/or amnesia of seizures, and intracranial EEG (iEEG) activity during 102 FIAS and 36 FAS from 35 human patients both in the seizure onset zone (SOZ) and areas remote from SOZ. We first compared iEEG slow-wave activity (SWA, i.e. delta power, 1 -

4 Hz) and beta/delta ratio (a validated marker of cortical arousal), and further high gamma power (80 - 150 Hz) and high gamma phase-locked to lower frequencies (phase-locked high-gamma [PLHG]; a marker of ictal recruitment), and their evolution from the first to the second half of seizures.
While delta power was increased in most brain regions during FIAS, it only increased in the SOZ during the second half of the seizure for FAS. Interestingly, we however observed widespread decreases in beta-delta ratio in all areas outside of the SOZ (frontal, temporal, and parieto-occipital regions) not only in FIAS, but also in FAS ($p<0.01$ for all reported comparisons). During FIAS, PLHG increased in SOZ and temporal lobe for FIAS, while it showed a whole-brain decrease during FAS. Similarly, high gamma power increases against baseline ($p<0.01$) were found in all brain areas during FIAS - while it decreased across the whole brain during FAS. Both PLHG and high gamma features were found to be higher in FIAS compared to FAS. Taken together, these results suggest that sleep-like activities may be present not only during FIAS but also, to a smaller degree, during FAS. Such sleep-like activities may account for milder symptoms such as distractedness or attenuated perception of the environment, that many patients describe during FAS seizures. Future studies will investigate differences in cortical topography associated with LOC and directly compare ictal sleep-like activities to nocturnal sleep iEEG in the same patients.

Disclosures: U. Gorska: None. **C. Kozma:** None. **C. Papantonatos:** None. **M. Grobbelaar:** None. **E. Juan:** None. **A. Struck:** None. **H. Blumenfeld:** None. **G. Tononi:** None. **M. Boly:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.05

Topic: B.10. Epilepsy

Support: Stanford Wu Tsai Neurosciences Institute
Stanford Bio-X Seed Grant IIP-104

Title: Seizure detection in human intracortical electroencephalography using joint entropy estimations

Authors: *L. YAMADA[1], T. OSKOTSKY[2,3], P. NUYUJUKIAN[2,3,1,4,5];
[1]Electrical Engin., [2]Bioengineering, [3]Neurosurg., [4]Wu Tsai Neurosciences Inst., [5]Bio-X Inst., Stanford Univ., Stanford, CA

Abstract: Of the 1% of the world population with epilepsy, one-third have refractory epilepsy and often turn to surgical options. The clinical standard of epilepsy treatment relies on manual

review of electroencephalography (EEG) by an epileptologist and could benefit from reliable quantitative EEG (qEEG) approaches to speed up evaluation, minimize inter-reviewer variance, and deliver better care. Here we present the inverse compression ratio (ICR), a method to estimate an unbiased upper bound of joint entropy as a potential qEEG method for seizure detection. Using our data repository of 10 kHz intracortical neuroelectrophysiology across 30 participants (17 adults and 13 children, 249 total seizures), single-electrode ICR achieved an area under Receiver Operating Characteristic (ROC) curve of 0.99. Further, multielectrode ICR performed similarly to, or better than, existing qEEG methods, eliminating the need to process each electrode independently. To the best of our knowledge, this represents the first demonstration of ICR as a multidimensional estimator in biological or clinical applications. By computing an ensemble effect without imposing linearity or data structural assumptions, ICR offers an unbiased, model-free solution to the classically combinatorially intractable problem of high-dimensional joint entropy; its application may extend beyond epilepsy to other domains of biomedical signal processing.

Disclosures: L. Yamada: None. **T. Oskotsky:** None. **P. Nuyujukian:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.06

Topic: B.10. Epilepsy

Support: NIH/NINDS R01NS094743

Title: Hippocampal dentation based differentiation of healthy controls and epileptic subjects using high resolution MR images

Authors: *A. KILPATTU RAMANIHARAN, G. SELLADURAI, L. VER HOEF;
Neurol., The Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Hippocampal dentation (HD) is a complex morphological feature seen on the inferior aspect of human hippocampi that is highly heterogeneous across healthy controls (HC) and is positively correlated with memory performance. Previously we quantified HD intemporal lobe epilepsy (TLE) patients using a subjective method on T1w images and found that HD was significantly less in epileptic than contralateral hippocampi. However, the segmentation of hippocampi using standard T1w images under-estimates the depth of prominent dentes on the 3D surface rendering and subjective HD quantification is prone to inconsistency across raters. In this work, we use a novel objective HD quantification method to measure the degree of HD in HC and TLE groups using T2w High Resolution Multiple Image Co-Registration and Averaging (HR-MICRA) images. T1w and HR-MICRA images were acquired from 26 HC and 23 TLE

patients. The images were resampled to ~0.25mm isotropic. Segmentation of hippocampi was performed using Automatic Segmentation of Hippocampal Subfields software. Surface meshes were generated from the segmentations. The objective method quantifies HD by transforming a strip of a 3D surface mesh into a dimensionally reduced and lowpass filtered 2D plot followed by area-under-the-curve (AUC) calculation. Student's t-Test was used to compare the AUC values obtained using T1w and HR-MICRA images. Asymmetry in HD was calculated by subtracting the AUC values of right hippocampi from the left. One-way ANOVA was performed with Tukey post-hoc test to differentiate the asymmetry in HD between HC, left-TLE and right-TLE groups. HR-MICRA images had higher AUC values compared to T1w images ($p = 0.03$). Among HC, the AUC values were significantly greater in the left compared to their right hippocampi ($p = 0.001$). A one-way ANOVA yielded significant difference in HD among the groups, ($F(2, 46) = [3.33]$, $p = 0.04$). A post hoc Tukey test showed that HD asymmetry differed significantly between left and right TLE groups ($p < 0.05$) but not HCs with a shift in HD away from the epileptic side. Using a novel objective measurement of HD, we conclude the following: HR-MICRA images capture complex surface undulations of HD more effectively than T1w images, indicated by lower T1w AUC values; Normal individuals show a greater degree of HD (i.e.higher AUC values) in the left hippocampus than on the right, likely due to greater degree of functional specialization in the left hemisphere; There is a significant difference in the asymmetry of HD between left and right TLE, indicating a loss of HD on the epileptic side.

Disclosures: A. Kilpattu Ramaniharan: None. **G. Selladurai:** None. **L. Ver Hoef:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.07

Topic: B.10. Epilepsy

Support: NIH Grants NINDS U54 NS100064, The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx)
Submission on behalf of the EpiBioS4Rx investigators

Title: Fully-automated MRI-based machine learning classification of patients with and without epileptic seizures following traumatic brain injury

Authors: *I. YILDIZ[1], R. GARNER[1], P. VESPA[2], D. DUNCAN[1];
[1]Keck Sch. of Medicine, USC Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA; [2]David Geffen Sch. of Med., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Traumatic brain injury (TBI) is a prevalent cause of severe disorders, including post-traumatic epilepsy (PTE). PTE is diagnosed if seizures occur at least one week post-TBI, though

they can emerge even in years after a TBI (Diaz-Arrastia et al., 2009). The unpredictable progress of PTE motivates early identification of epileptic biomarkers. The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) is a multi-site project aiming to detect epileptogenesis and initiate antiepileptogenic therapy after a TBI (Duncan et al., 2018b). Magnetic resonance imaging (MRI), with particular focus on structural MRI (sMRI), has been commonly employed due to its non-invasive nature (Lutkenhoff et al., 2020). Recent methods using sMRI apply machine learning (ML) to distinguish patients who develop at least one late seizure following a TBI from those who do not experience any (La Rocca et al., 2020). Nevertheless, existing works require several preprocessing steps to extract correlation-based features predicting seizure, further cleaned from noise and non-brain voxels. To the best of our knowledge, we propose the first ML method that directly ingests sMRI without manual feature extraction and cleaning, excluding registration and skull stripping. Our work is inspired by the success of neural networks in image classification, including MRI (Bien et al., 2018). Our neural network architecture involves three branches, each automatically learning features on one of the axial, sagittal, and coronal planes. Features learned from all branches are concatenated and used to classify each MRI scan as seizure-positive or not. Our sMRI dataset contains 79 good quality scans, of which 17 are seizure-positive. We partition the dataset into training, validation, and test sets of 60%, 20%, and 20% portions, respectively, in a stratified manner. We augment seizure-positive training scans to combat class imbalance. The architecture is trained and validated to minimize classification error. We evaluate the architecture along with 95% confidence interval (Wilson, 1927) on the holdout test set. Our method attains 0.87 +/- 0.16 accuracy, 0.93 +/- 0.12 specificity, 0.66 +/- 0.23 sensitivity, and 0.64 +/- 0.23 Area Under the receiver-operating-statistics curve (AUC). In this work, we take the first successful steps of fully-automated MRI-based classification of patients with and without seizures, aiming to ease the time and effort involved in epileptogenesis biomarker identification after a TBI. Early and accurate detection of epileptogenesis via our method could facilitate successful clinical trials of medication development and antiepileptogenic therapy.

Disclosures: I. Yildiz: None. **R. Garner:** None. **P. Vespa:** None. **D. Duncan:** None.

Poster

P155. Epilepsy: Seizure Detection and Propagation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P155.08

Topic: B.10. Epilepsy

Support: NSF Graduate Research Fellowship 1747505
NIH NINDS K23 NS114178
NIH UH3 NS109557

Title: Cohort analysis of activation volumes and responsive neurostimulation parameters in patients with mesial temporal lobe epilepsy

Authors: *C. M. CHARLEBOIS[1], D. N. ANDERSON[2], K. A. JOHNSON[6], B. J. PHILIP[3], B. J. NEWMAN[4], A. Y. PETERS[4], A. M. ARAIN[4], A. D. DORVAL[3], J. D. ROLSTON[5], C. R. BUTSON[7];
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Abstract: Responsive neurostimulation (RNS) is a closed-loop, FDA-approved treatment for patients with refractory epilepsy. Most patients receiving RNS therapy experience a decrease in seizure frequency over several years. However, clinical outcomes are variable across patients. It is currently unknown how to tailor stimulation to provide seizure freedom. Our objective was to quantify the brain regions stimulated and the changes in stimulation parameters following RNS implantation. We collected retrospective pre-operative MRI and diffusion-weighted imaging, post-operative CT, stimulation settings, and clinical outcomes for 31 patients receiving mesial temporal RNS at the University of Utah. We registered pre- and post-operative imaging to identify contact locations and estimated patient-specific volumes of tissue activated (VTA) based on the latest stimulation setting. To compare stimulation across the cohort, we warped the VTA to a common atlas and a single hemisphere. Active contacts were distributed across the amygdala and hippocampus (Fig 1A). Patients usually began treatment with low current densities that increased over time (Fig 1B) based on programming guidelines from NeuroPace. The stimulation amplitude changed an average of 2.33 times per year and the average time to change stimulation montage was 2.48 years. The region of greatest VTA overlap across patients was the anterior hippocampus (Fig 1C). For most patients, clinicians increased the current density over time, modulating larger regions of the temporal lobe. However, the patients who became seizure free responded sooner and with consistently lower current density. These initial results suggest that seizure responsiveness in the first year after implantation correlates with long-term symptom relief. Further, ineffective seizure suppression in the first year may be associated with a protracted series of parameter adjustments yielding an eventual decrease in seizures over years. In ongoing work, we plan to identify predictors of seizure freedom and programming strategies that decrease response time.

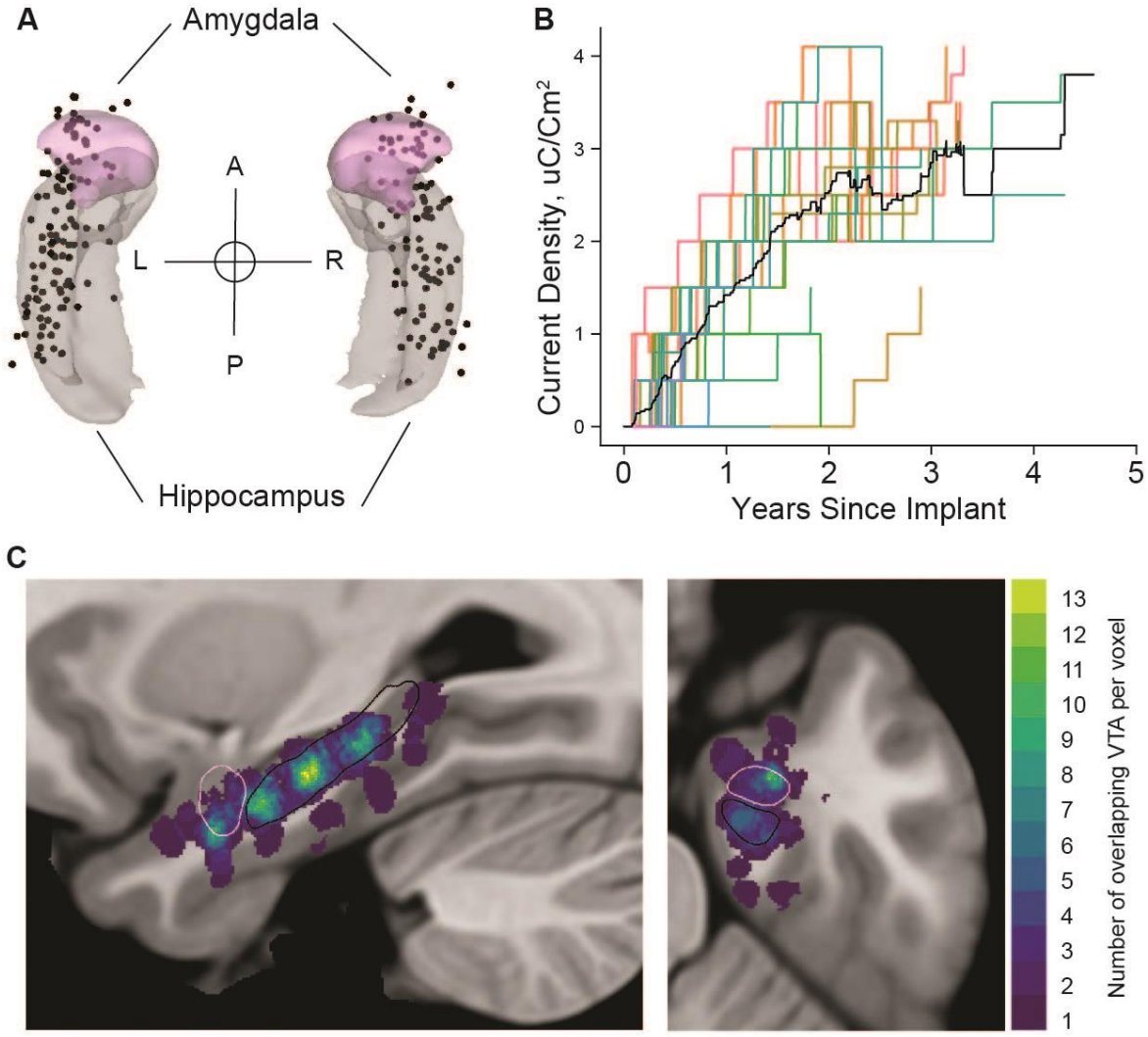


Figure 1. *A. Active Contact Locations Across Patients.* Superior view of the center of each stimulation contact implanted near the mesial temporal lobe across 31 patients (n=220 contacts). *B. Stimulation trends over time.* Each colored trace is the current density of stimulation for a single patient over time. The black trace shows the mean current density over all 25 patients currently treated with stimulation. *C. Neural Activation Patterns.* The left shows a sagittal slice of the overlapping volumes of tissue activated (VTA) within the mesial temporal lobe and the right an axial slice for each subject's most recent stimulation settings (n=23 patients). The VTA of both hemispheres have been mapped to a single hemisphere (n=42 VTA). The majority of leads implanted have 10 mm spacing (40/42). This lead spacing spread activation across the long axis of the hippocampus with maximal overlap throughout the middle of the hippocampus and within the amygdala.

Disclosures: C.M. Charlebois: None. **D.N. Anderson:** None. **K.A. Johnson:** None. **B.J. Philip:** None. **B.J. Newman:** None. **A.Y. Peters:** None. **A.M. Arain:** None. **A.D. Dorval:** None. **J.D. Rolston:** F. Consulting Fees (e.g., advisory boards); Medtronic, Inc., Corlieve Therapeutics. **C.R. Butson:** F. Consulting Fees (e.g., advisory boards); Abbott, NeuraModix.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.01

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

Support: NIRG-12-241456
NIH-NIGMS-5P20GM103446

Title: Overexpression of the small heat shock protein 27 reduces aggregation of transactive response DNA binding protein 43

Authors: *M. I. ABEER, M. A. GITCHO;
Delaware State Univ., Dover, DE

Abstract: Transactive response DNA binding protein 43 (TDP-43) is the major pathological protein in both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). ALS is a progressive neurodegenerative disease causing muscle weakness and a loss of voluntary muscle movements due to degeneration of motor neurons in the brain and spinal cord leading to death. FTD is the second most frequent cause of dementia accounting for ~10% of all dementia cases. Recently, pathological TDP-43 was discovered as a secondary pathology in up to 50% of Alzheimer's disease (AD) cases. TDP-43 was previously shown to bind to heat shock protein family B (small) member 1 (HSPB1 or HSP27) but no functional evaluation of this interaction has been explored. HSP27 consists of an alpha-crystallin domain that is involved in oligomerization and acts as a molecular chaperone for refolding proteins. In response to stress, HSPs work to help fold native proteins in order to reduce aggregation. Inducing expression of HSP27 has been shown to be protective of acute rejection after cardiac transplant, diabetic sensory neurodegeneration protects in a model of spinocerebellar ataxia type 17, and has been shown to reduce aggregation of amyloid. In the present study, we examine the effect of HSP27 overexpression on TDP-43 aggregation and mitochondrial morphology. A new transgenic mouse model was developed to selectively drive human HSP27 and pathological TDP-43 with a defective nuclear localization signal (ΔNLS) in the hippocampus and neocortex using the Ca^{2+}/calmodulin protein kinase (Camk2a) tetracycline-inducible system. We evaluated the following genotypes: wild-type, Camk2a/NLS, Camk2a/HSP27, and Camk2a/HSP27/TDP43ΔNLS at 4 months of age for immunohistochemistry, biochemistry (solubility fractionation), and Western blot. Preliminary *in vitro* results show that cells overexpressing HSP27 reduce aggregation and protein levels of TDP43. Mice overexpressing HSP27 in a TDP43ΔNLS background show a reduction of aggregated TDP43. We also examined protein changes in full-length TDP43, N-terminal and C-terminal fragments of TDP-43, HSP27, and apoE4. Further experiments will be carried out to investigate the expression of ubiquitin, glial fibrillary acidic protein (GFAP), synapsin-1, and ubiquitin-binding protein p62 to characterize this new model of neuronal protection. Immunohistochemical and bioenergetic experiments will be carried out to evaluate the overall brain and mitochondrial morphology upon

HSP27 overexpression. Our initial preliminary data suggests that modifying HSP27 expression may provide a point of therapeutic intervention for TDP-43 proteinopathies.

Disclosures: M.I. Abeer: None. **M.A. Gitcho:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.02

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

Support: NIH Grant R01AG06115
NIH Grant R01AG055523.
DH Chen Foundation R-86U55A

Title: Normal levels of KIF5 but reduced KLC1 levels in both Alzheimer disease and Down syndrome

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Abstract: Impaired axonal transport may contribute to the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD) and Down syndrome (DS). Axonal transport is a complex process in which specific motor proteins move cargoes to and from neuronal cell bodies and their processes. Inconsistent reports point to the changes in AD in the levels of the classical anterograde motor protein kinesin family member 5 (KIF5) and the primary neuronal KIF regulator kinesin light chain 1 (KLC1), raising the possibility that anterograde transport is compromised in AD. We measured the levels of all the three KIF5 family members and KLC1 in the AD and AD-DS frontal cortex and AD temporal cortex and cerebellum in samples taken with a short postmortem interval and in the brains of young and aged adult mice in the Dp (16)1Yey/+ (Dp16) mouse model of DS and J20 mouse model of AD. There were no changes in comparison with controls in all the three KIF5 family members in either the AD or AD-DS samples when normalized to either β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Interestingly, however, samples from control brains as well as from AD and AD-DS demonstrated strong positive correlations between the levels of KIF5 family members. In contrast to the findings for KIF5, the levels of KLC1 were downregulated robustly in the frontal cortex of both AD and AD-DS brains; interestingly, this change was not seen in the AD temporal cortex or cerebellum. Studies comparing Dp16 to its euploid control recapitulated human studies in demonstrating no change in KIF5 levels. J20 mice also showed normal KIF5 levels. However,

unlike the AD and AD-DS frontal cortex, KLC1 levels were not reduced in the brains of Dp16 or J20 mice. Together, there are significant reductions in the levels of KLC1 but not KIF5 family members in AD and AD-DS, implying compromised KLC1-mediated axonal transport in these conditions.

Disclosures: X. Chen: None. **U. Das:** None. **W.C. Mobley:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.03

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

Support: R01 NS104282

Title: Progression of Depression-Associated Behaviors in a Mouse Model of Alzheimer's Disease Following Traumatic Brain Injury

Authors: *J. IANNUCCI, S. BEEVERS, K. PULIDO, V. ARISMENDI, L. VENKATASAMY, M. A. HOOK, F. SOHRABJI, L. A. SHAPIRO;
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Abstract: Background: Traumatic brain injury (TBI) is a substantial risk factor for developing Alzheimer’s disease (AD) and related dementia. Depression occurs in 50-66% of TBI patients, and has also been shown to be a major predictor of dementia, AD, and AD-related dementia. Here, we investigated the progression of a depression-associated phenotype in both WT and AD animals following TBI. We hypothesized that exposure to TBI would worsen performance in our depression behavior battery, and that this would progress over time. **Methods:** Male WT and 5xFAD animals were subjected to a depression battery consisting of the sucrose preference test (SPT), social interaction test, and burrowing test. Animals were tested once prior to TBI to establish a baseline. WT and 5xFAD animals then underwent surgery where they received either sham or fluid percussion injury (FPI). All animals were then tested with the same depression battery one week after injury, and then once a month for the next 4 months post-injury to evaluate progression of a depression-associated phenotype. **Results:** We found that 5xFAD mice exhibited significant differences in depression-associated behaviors compared to WT prior to injury, including significantly reduced burrowing in the burrowing test and altered social interaction. Following TBI, we observed a change in performance on depression-associated behaviors in both WT and 5xFAD animals, with particular differences observed in increased social avoidance and decreased burrowing. The effects of TBI were more pronounced in 5xFAD mice compared to WT. **Conclusions:** Our results demonstrate the connection between TBI and increased depression-associated behaviors. These findings additionally highlight the potential

link between TBI and AD by suggesting a common pathological feature, and indicating that TBI may exacerbate pre-existing pathology in AD.

Disclosures: J. Iannucci: None. **S. Beevers:** None. **K. Pulido:** None. **V. Arismendi:** None. **L. Venkatasamy:** None. **M.A. Hook:** None. **F. Sohrabji:** None. **L.A. Shapiro:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.04

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

Support: NIA AG033570
NIA G033570-S1,S2
NIA AG06023
NIA AG062251
NIA AG061628 (O.L.)
Rush University Memory and AgingProject (UH2NS100599, D.A.B.

Title: Adult human hippocampal neurogenesis in aging and disease

Authors: *A. DISOUKY[1], M. TOBIN[1], W. G. HONER[2], K. ARFANAKIS[3], N. KIM[3], A. SHETTI[4], R. DAWE[3], D. A. BENNETT[3], O. LAZAROV[5];
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Abstract: The generation of newly born neurons in the hippocampus of rodents during adulthood has been widely accepted. This phenomenon, known as adult hippocampal neurogenesis, has been well characterized in various rodent models and has been shown to play an important role in learning and memory. However, the existence and extent of neurogenesis in the adult human hippocampus has been a topic of debate in recent years. Here, we provide evidence for the persistence of neurogenesis in the hippocampus of healthy Aging humans up to the tenth decade of their life. Moreover, we observed the presence of new neurons in the brains of patients with mild cognitive impairments (MCI) and Alzheimer’s disease (AD). We further show a significant reduction of the number of neuroblasts and new neurons in these patients, which may suggest that neurogenesis is compromised in an early stage of cognitive deterioration. In addition, the number of newly generating neurons correlates with the expression of key presynaptic proteins, previously shown to be associated with cognitive reserve. Interestingly, we observed that higher numbers of neuroblasts significantly correlated with better cognitive z-

scores, suggesting that individuals with greater numbers of neuroblasts are less likely to experience cognitive decline. Lastly, we show that the neural progenitor cells, neuroblasts, and immature neurons were distributed throughout the ventral-dorsal axis of the human hippocampus. Taken together these data suggest that hippocampal neurogenesis persists in the brain throughout life, but is compromised in AD and that enhancing neurogenesis may support cognitive function in AD.

Disclosures: A. Disouky: None. **M. Tobin:** None. **W.G. Honer:** None. **K. Arfanakis:** None. **N. Kim:** None. **A. Shetti:** None. **R. Dawe:** None. **D.A. Bennett:** None. **O. Lazarov:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.05

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

Support: NIH grant R01 NS084817
NIH grant R01 DA044552
NIH grant R01 DA044552-03S1
NIH grant R01 DA033966
NIH grant R01 NS060632

Title: Medial prefrontal cortex pyramidal neurons exhibit hyperexcitability and synaptic dysregulation at early stage of Alzheimer's Disease modeled in 3xTg-AD mice

Authors: *N. CHOUDHURY, L. CHEN, V. T. NGUYEN, L. AL-HARTHI, **X.-T. HU**; Microbial Pathogens and Immunity, Rush Univ. Med. Ctr., Chicago, IL

Abstract: The brain regions that show an early increase of beta-amyloid (Aβ) in Alzheimer's disease (AD) overlap with the default mode network (DMN), which comprises, but is not limited to, the medial prefrontal cortex (mPFC), a key regulator of neurocognition. DMN is active during mental processes that don't require attention, but it is deactivated when undertaking neurocognitive tasks. However, such DMN deactivation is impaired in AD, often preceding the formation of the Aβ plaque and the onset of clinical symptoms. Previous studies suggest that neuronal hyperexcitability is responsible for increased DMN vulnerability to early Aβ deposition. However, possible underlying neuronal dysfunction in critical DMN brain areas, like mPFC, is unknown and has not been studied. Here we used young 3xTg-AD mice (3-5 months of age) to mimic the early stages of AD prior to the formation of cortical Aβ plaques. Patch-clamp electrophysiology was applied to assess functional activity of mPFC pyramidal neurons in brain slices from 3xTg-AD mice and it was compared with wild-type (WT) mice. Firing was evoked by moderate depolarizing currents that mimicked physiological excitatory inputs. Spontaneous

excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively) were assessed at gap-free holding potential of -70 mV and 0 mV, respectively. Ca^{2+} spikes (indicating Ca^{2+} influx through voltage-gated Ca^{2+} channels, VGCCs) were evoked by membrane depolarization with blockade of voltage-gated Na^{+}/K^{+} channels, ionotropic glutamate and $GABA_A$ receptors. We found that firing of mPFC neurons was significantly increased, which was associated with altered membrane properties (i.e., depolarized resting membrane potential, decreased rheobase, increased input resistance, and reduced firing threshold), and significantly increased frequency of sEPSCs in young 3xTg-AD mice. Selective blockade of NMDA or AMPA receptors (R) eliminated the increased sEPSCs. There was no significant change in sIPSCs mediated by $GABA_A$Rs or Ca^{2+} spikes mediated by VGCCs in 3xTg-AD mice. Together, these novel findings demonstrate that the excitability of mPFC pyramidal neurons is aberrantly increased at the early stages of AD preceding Aβ plaque formation accompanied by enhanced glutamate neurotransmission mediated by NMDARs/AMPARs. Whether $GABA_A$R or VGCC activity will be altered at older age needs further investigation. Based upon these findings we propose that this initial mPFC neuronal hyperactivity could trigger consequential dysregulation in the brain that ultimately results in Aβ plaque formation. Further study is required to confirm or disconfirm it.

Disclosures: N. Choudhury: None. **L. Chen:** None. **V.T. Nguyen:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.06

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

Support: National Institute of Neurological Disorders and Stroke (NS085770)
Alzheimer’s Disease Center Grant from the National Institute on Aging (AG013854)

Title: Ubiquitin Immunoreactivity Identifies TDP-43 Inclusions and Marks Cortical Neurons Destined to Degenerate in a Mouse Model of Frontotemporal Lobar Degeneration

Authors: *A. CHIODINI[1], I. A. AYALA[1], **C. GEULA**[1,2], M.-M. MESULAM[1,3], K. SADLEIR[3], R. VASSAR[3];
[1]Mesulam Ctr. for Cognitive Neurol. and Alzheimer's Dis., [2]Dept. of Cell & Developmental Biol., [3]Ken and Ruth Davee Dept. of Neurol., Feinberg Sch. of Med. at Northwestern Univ., Chicago, IL

Abstract: Accumulation of TDP-43 inclusions is one of the pathological hallmarks of frontotemporal lobar degeneration (FTLD). Mouse models have shown that overexpression of wild-type or mutant human TDP-43 (hTDP-43) can result in the formation of inclusions and neuronal loss. In the human brain, TDP-43 inclusions are strongly ubiquitinated. The purpose of this study was to investigate ubiquitination within cortical neurons in a conditional transgenic mouse model expressing wild-type hTDP-43 under the control of tetracycline operator sequences (tTA). tTA and hTDP-43 transgenic mice were bred on 129SVE and FVB backgrounds respectively. Double transgenic pups were weaned at 21 days of age, at which time expression of TDP-43 was initiated. Brains were examined after 14 days, 8 weeks and 24 weeks of TDP-43 expression. Consistent with our previous findings, small round intraneuronal inclusions containing phosphorylated TDP-43 were observed in many cortical areas after 14 days of TDP-43 expression. After 8 weeks, the number of these inclusions was significantly reduced, and by 24 weeks, no inclusions were observed. This course of events matches the progression of cortical atrophy and decreased density of neurons we observed in these animals at 8 and 24 weeks of expression. After 14 days of expression, ubiquitin immunoreactivity visualized small round cytoplasmic inclusions identical to those observed with phosphorylated TDP-43 staining. In addition, intense ubiquitin immunoreactivity was observed in a subset of cortical neurons. At 8 weeks of TDP-43 expression, ubiquitin stained inclusions and neurons displayed a substantial decrease, and by 24 weeks none could be identified. Thus, as is the case in FTLD, TDP-43 inclusions is transgenic mice are highly ubiquitinated. Furthermore, prominent cytoplasmic ubiquitin immunoreactivity is present in neurons that contain inclusions. Ubiquitin immunoreactivity appears to mark neurons that are destined to degenerate, resulting in disappearance of inclusions within them and cortical atrophy.

Disclosures: A. Chiodini: None. **I.A. Ayala:** None. **C. Geula:** None. **M. Mesulam:** None. **K. Sadleir:** None. **R. Vassar:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.07

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

Support: NIRG-14-321390
ADHS16-104646
FY2016
Arizona Alzheimer's Consortium
SOLUR Program

Title: Mislocalized expression of the nuclear pore complex proteins NUP153, -93, and -214 in Alzheimer's disease brains

Authors: *M. GORAS, D. BROKAW, E. DELVAUX, P. COLEMAN;
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Abstract: Alzheimer's disease (AD) is a progressive, neurocognitive disorder characterized by memory dysfunction. The presence of neuropathological aberrations, namely amyloid plaques, and neurofibrillary tangles, although key characteristics of this disease, have shown to be poor prognostic indicators. As such, clinical trials targeting AD neuropathology have largely been unfruitful, necessitating new research into novel mechanisms of the underlying pathways mediating cognitive decline. Nuclear pore complexes (NPCs) are the main conduits for molecular exchange across the nuclear envelope in eukaryotic cells. The NPC contains approximately 30 distinct nucleoporins (NUPs) which form a selective channel that supports the factor mediated shuttling of cargo through the NPC. Mutations in nucleoporin genes have been linked to various human diseases including neurodegenerative diseases. Tau is the major component of neurofibrillary tangles in (AD), and a recent study has suggested a role in NPC deterioration and thus nuclear cytoplasmic defects. This study however was not an extensive one and only investigated four nucleoproteins from one layer of the NPC structure. In this study, we have targeted all three major components of the NPC structure, by analyzing gene expression of representative NUPs from homogenate brain tissue and neuronal data in AD brains. Three significantly differentially expressed NUPs representing different parts of the NPC structure were selected for validation by immunohistochemistry and Western blotting in postmortem human hippocampal sections. Bioinformatic analysis revealed widespread differential NUP gene expression across multiple brain regions in AD. Our findings revealed mislocalization of cytoplasmic-facing nucleoporin NUP214 in the cytoplasm in AD and nuclear localization of inner ring and nuclear basket nucleoporins NUP93 and NUP153 in hippocampal CA1 neurons. These results and this research, represents one of the first attempts to categorize differential changes throughout the entire structure of the NPC in AD brains. Future studies will explore the hierarchical relationship between neuropathological hallmarks of AD and NPC aberrations to better understand the etiology of impaired nucleocytoplasmic transport in neurodegeneration.

Disclosures: M. Goras: None. **D. Brokaw:** None. **E. Delvaux:** None. **P. Coleman:** None.

Poster

P240. Proteinopathy and Pathology Other Than Abeta/Tau

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P240.08

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

Support: NIA RF1 AG059392

NIA T32 AG055378
NIH UH2/UH3 TR000903
NIA P50 AG08017

Title: Mir-193a-5p as a regulator of intersectin1-short in Alzheimer's disease

Authors: *S. J. SMITH, J. A. SAUGSTAD;
Anesthesiol. & Perioperative Med., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Alzheimer's disease (AD) is the most common form of dementia and is characterized by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles in the brain. Currently, the pathogenesis underlying AD has not been established, and there are no reliable tools to detect early changes in the AD brain. Our previous studies revealed a set of microRNAs (miRNAs) that are decreased in cerebrospinal fluid (CSF) of AD patients and that serve as biomarkers to distinguish AD from neurologically normal age-matched controls. MiRNAs regulate post-transcriptional gene expression through complementary binding to target mRNAs, which largely results in repressed mRNA translation. However, which mRNAs are targeted and regulated by the AD CSF miRNAs, and how these may affect proteins involved in AD pathophysiology, is not yet known. We developed a bioinformatics pipeline to predict mRNA targets of the AD miRNA biomarkers, and identified Intersectin1-short (*ITSN1-s*) mRNA as one prominent target of the AD CSF miRNA, miR-193a-5p. ITSN1-s protein is associated with multiple pathways relevant to AD, however no studies to date have assessed the role of miRNAs or miR-193a-5p in the regulation of ITSN1-s in AD. Immunoblot analysis of post-mortem human hippocampal tissues show a significant increase in ITSN1-s protein in both male and female AD patients (n = 34) as compared to age-matched controls (n = 23). As ITSN1-s is expressed in multiple brain cell types, we also evaluated how cell type-specific expression of ITSN1-s may change in AD brains, and how altered miR-193a-5p levels may affect these changes. We examined which cell types express miR-193a-5p and ITSN1-s mRNA through miRNAscope and RNAscope paired with immunohistochemistry stains for cell type markers in AD (n = 10) and control (n = 10) post-mortem hippocampal sections. These analyses show that ITSN1-s is increased in specific brain cell types and miR-193a-5p is also changed. Additionally, we have confirmed that miR-193a-5p directly binds and regulates ITSN1-s mRNA translation through luciferase assays using HT-1080 cells (n=8), along with paired RT-qPCR and immunoblots of ITSN1-s mRNA and protein after addition of miR-193a-5p mimic to HEK293t cells (n = 8).

Disclosures: S.J. Smith: None. **J.A. Saugstad:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.01

Topic: C.03. Parkinson's Disease

Support: DFG ME 4197

Title: Glutamatergic neurotransmission in the inferior colliculus affects lateralization in hemi-parkinsonian rats

Authors: *R. K. W. SCHWARTING[1,2], **L. MELO-THOMAS**[1,3];
[1]Philipps-University Marburg, Marburg, Germany; [2]Ctr. for Mind, Brain and Behavior (CMBB), Marburg, Germany; [3]Behavioral Neurosciences Inst. (INeC), Ribeirão Preto, Brazil

Abstract: Rats with 6-OHDA unilateral lesion of the medial forebrain bundle (MFB) have been used as a model for Parkinson's disease (PD). After lesion, rats exhibit lateralized deficits in spontaneous behavior, such as ipsiversive (towards the lesioned side) rotations. After systemic challenge with the dopamine D2 receptor agonist apomorphine, they exhibit contraversive (towards the non-lesioned side) rotation. The aim of the present study was to investigate whether such lateralization is attenuated by glutamatergic mechanisms in the inferior colliculus (IC), an auditory structure in the brainstem with output connections to motor pathways and movement coordination systems. For that, adult male Wistar rats received unilateral microinjection of 6-OHDA (lesion group) or saline (sham lesion group) into the MFB and microinjection cannulas were implanted bilaterally in the IC. The week later, rotation was assessed before (pre APO condition) and after apomorphine administration (post APO condition). In the pre APO condition, the spontaneous ipsiversive asymmetry disappeared after microinjection of the glutamatergic antagonist, MK-801, into the IC irrespective of the IC microinjection side (ipsi-, contra- or bilaterally to the lesion). Such an absence of asymmetry was also observed when the glutamatergic agonist NMDA was microinjected into the IC contralateral to lesion. NMDA microinjected into the ipsilateral IC produced a clear contraversive asymmetry, i.e. reverted the lateralization. In the sham lesion group, there was no effect of glutamatergic IC treatment. The contraversive asymmetry observed during the post APO condition was potentiated by MK-801 microinjected into the IC contralateral to lesion side, whereas it was unaffected when this microinjection was performed bilaterally or into the ipsilateral IC. Also, NMDA microinjected into the IC ipsi- or contralateral to lesion side did not affect contraversive asymmetry induced by APO in lesioned rats. These results support our hypothesis that the hypersensitivity of the lesioned striatum can be influenced by a glutamatergic intracollicular substrate. Further, the present data suggest that the side of the IC microinjection with respect to the lesion is particularly important. Understanding of how glutamatergic neural substrates in the IC influences motor control, classically attributed to the basal ganglia circuitry, can be useful in the development of new therapeutics to treat PD and other motor disorders.

Disclosures: R.K.W. Schwarting: None. **L. Melo-Thomas:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.02

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: The effects of L-DOPA-induced motor and non-motor side effects in a bilateral rat model of Parkinson's disease

Authors: *N. LIPARI[1], A. CENTNER[2], S. COHEN[2], J. GLINSKI[2], C. R. BISHOP[3];
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Abstract: Parkinson's Disease (PD) is most commonly characterized by severe dopamine (DA) depletion within the substantia nigra (SN) leading to a myriad of motor and non-motor symptoms (NMS) namely, cognitive and affective dysfunction. Currently, the gold-standard treatment for late-stage PD utilizes levodopa (L-DOPA) to replenish DA loss thereby restoring motor function, however, its chronic use induces side effects like L-DOPA-induced dyskinesia (LID) and may even exacerbate some NMS. Indeed, anxiety and Parkinson's disease associated psychosis (PDAP), deleteriously impact the quality of life for PD patients, particularly in later stages of the disease. Unfortunately, they and their mechanisms remain vastly understudied. To examine L-DOPA-induced motor and non-motor side effects, this study employed a bilateral 6-hydroxydopamine (6-OHDA) lesion or sham lesion in male and female Long Evans rats (N=48). Thereafter, subjects were administered daily vehicle as control or L-DOPA (6mg/kg, s.c.) to establish LID while examining treatment-related effects on prepulse inhibition (PPI), a measure of sensorimotor gating, open field, to examine anxiety, abnormal involuntary movements (AIMs) for LID and forepaw adjusting steps (FAS) to examine lesion-induced deficits in movement. Bilaterally lesioned animals treated with L-DOPA showed the development of mild to moderate dyskinesia, with considerable variability while also exhibiting modest but significant L-DOPA-related improvement on the FAS test. Results suggest L-DOPA may also affect sensorimotor gating which was predicted in part by AIMs severity. Finally, lesioned, L-DOPA-treated rats exhibited an increase in anxiety-like behaviors. Altogether, this project established a bilateral 6-OHDA lesion rat model that displayed motor and nonmotor deficits and treatment-related side effects. By extension, this bilateral lesion approach may yield important information on symptom progression and the mechanisms that underlie them.

Disclosures: N. Lipari: None. **A. Centner:** None. **S. Cohen:** None. **J. Glinski:** None. **C.R. Bishop:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.03

Topic: C.03. Parkinson's Disease

Title: Evaluation of the goma guar polymer to regulate intestinal motility in Hemiparkinsonism rat model

Authors: *G. REYNOSO GÁLVEZ, E. GARCÍA VALDÉS, S. GALAVIZ HERNÁNDEZ, V. GALLEGOS HERNÁNDEZ, E. A. RODRÍGUEZ PÉREZ, P. VERGARA ARAGÓN; UNAM, MEXICO CITY, Mexico

Abstract: Parkinson's disease (PD) is usually characterized by cardinal motor impairments. However, a range of nonmotor symptoms precede the motor-phase and are major determinants for the quality of life. To date, no disease modifying treatment is available for PD patients. The gold standard therapy of levodopa is based on restoring dopaminergic neurotransmission, thereby alleviating motor symptoms, whereas non-motor symptoms remain undertreated. Constipation is a problem that affects approximately 50-70% of people with PD due to alterations in the autonomic nervous system, which regulates involuntary movements of the digestive tract. In this regard, it has been reported that the administration of soluble and insoluble fiber improves the symptoms of idiopathic chronic constipation. Guar gum (GG) is a polymer of relatively recent use, derived from the seeds of the Cyamopsis tetragonoloba plant from the Leguminosae family. GG has proven to be effective in softening and improving faecal production and increasing the capacity of faeces load and sensation of faecal excretion. Amaranth grain has unique protein content. Due its composition, amaranth protein resembles the one found in milk products and it is very close to the ideal protein characteristics proposed by the Food and Agriculture Organization for human consumption. The purpose of this paper is to propose a product which chemical properties will contribute to increase the peristalsis.

Disclosures: G. Reynoso Gálvez: None. **E. García Valdés:** None. **S. Galaviz Hernández:** None. **V. Gallegos Hernández:** None. **E.A. Rodríguez Pérez:** None. **P. Vergara Aragón:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.04

Topic: C.03. Parkinson's Disease

Support: T32 AG020494
Dept of Defense grant X81XWH-19-0757

Title: Is the Pink1 knockout rat a translatable animal model to evaluate prodromal cognitive and motor symptoms of Parkinson's Disease?

Authors: *I. SOTO[1], V. A. NEJTEK[2], E. KASANGA[1], M. F. SALVATORE[3];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by a substantial loss of motor function and cognitive decline, which is a key non-motor symptom (NMS). Early and accurate disease detection is crucial to prevent or slow disease progression. Thus, establishing the signs of a subtle prodromal NMS symptom such as cognitive decline may indicate PD diagnosis and eventual motor decline. In a proof-of-concept study, we used the Pink1 knock-out (KO) rat in comparison to wild-type (WT) rats to longitudinally examine progressive motor decline against NMS using the novel object recognition (NOR) test. We hypothesized that Pink1 KO rats would demonstrate progressive degeneration in both locomotor and cognitive functioning,, with evidence of impaired NOR earlier than motor signs, as evaluated by locomotor activity in the open-field test (OFT). From 3-months (baseline) to 6-months old, Pink1 KO rats traveled 34% less distance and moved 28% slower during the OFT and this decline reached significance at seven months old. One month later KO rats continued to decline, with 54% less distance travelled and lower speed (69%)vs baseline. In the NOR task Pink1 KO rats showed an initial decline in discrimination score from baseline to 4-months old. This difference was not present at 5-months old, as KO rats consistently spent more time with the novel *and* familiar object compared to WT. We found no effect of habituation to the test in the first component with presentation of two identical objects, suggesting that WT recognized the familiarity of the novel object over time, whereas the KO did not. These results suggest KO rats may exhibit perseveration and memory impairment, indicating impaired executive function. In summary, Pink1 KO rats show a steady decline in speed and distance traveled starting at 6 months old, preceded by a potential deficit in learning as early as 4-months old. Our initial results suggest that the Pink1 KO rat may model the NMS of cognitive impairment and the eventual motor impairment seen in PD progression.

Disclosures: I. Soto: None. **V.A. Nejtek:** None. **E. Kasanga:** None. **M.F. Salvatore:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.05

Topic: C.03. Parkinson's Disease

Support: Branfman Family Foundation
#DoD EP200077
NIH U01 NS099697
NSF CBET-1752274
NIH CTSA
UL1TR002553

Title: Quantitative Analysis of Cerebellar Cortex Activity, Neurodegeneration, and Gait in Aged PINK1 Knockout Rats

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by gait dysfunction in the advanced stages of the disease. PD hallmarks include a decrease in stride length, run speed, and swing time; an increase in stride time, stance time, and base of support; dopaminergic degeneration in the basal ganglia; and cholinergic degeneration in the pedunculopontine nucleus (PPN). Gait disturbances in PD may be associated with cerebellar dysfunction. Loss of dopamine in the substantia nigra pars compacta (SNc) of the basal ganglia may result in abnormal brain activity in the cerebellum. A progressive animal model of PD is needed to understand and identify treatments for gait dysfunction. This study quantified progressive gait dysfunction in PTEN-induced putative kinase 1 knockout (P1KO) rats. Neurodegeneration and cerebellar cortical activity were investigated as potential underlying mechanisms. Male P1KO (n=4) and age-matched wild-type (WT) Long-Evans hooded (n=4) rats were implanted with a 61-channel microelectrocorticography array (μECoG) over the cerebellar cortex (anteroposterior: -10.6mm to -14.0mm and mediolateral ± 2.6mm). Electrodes were arranged in an 8 x 8 grid with 230 μm diameter spaced 420 μm apart. Open Ephys was used to record cerebellar activity at vermis lobules V and VI (a and b) at 8 months. Gait analysis using a runway system was performed at 5 and 8 months of age and included stride length, run speed, swing time, stride time, stance time, and base of support. Immunohistochemical analysis of tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) was performed at the 8 month timepoint. P1KO rats showed significant gait deficits at 5 months, but not 8 months. At 8 months a 15% loss of TH in the striatum, a 27% loss of TH-positive cells in the substantia nigra pars compacta, and no significant loss of ChAT-positive cells in the PPN was found. Spectral power in lobule VIb delta band (1.5-3Hz) significantly decreased, and increased in the theta/alpha (4-10Hz), beta (10-30Hz), and gamma (30-80Hz) bands in P1KO rats vs. WT rats. No differences were found between genotypes in power in lobules V and VIa for any frequency bands.These results suggest that dopaminergic cell loss but not cholinergic cell loss contributes to gait deficits in the P1KO model. Gait abnormalities improved at the 8 month timepoint compared to the 5 month timepoint suggesting compensatory mechanisms in the P1KO model. Pathological oscillatory activity in cerebellar vermis lobule VIb may contribute to gait dysfunction. Further

analysis is required to determine the suitability of the P1KO rat as a progressive model of gait abnormalities in PD.

Disclosures: V. DeAngelo: None. **J.D. Hilliard:** F. Consulting Fees (e.g., advisory boards); Medtronic Consulting. **C. Chiang:** None. **J. Viventi:** None. **G.C. McConnell:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.06

Topic: C.03. Parkinson's Disease

Support: 1R25DK113652

Title: Apathy-like Deficits in a Rat Model of Early Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by motor and non-motor dysfunction. Apathy, or a loss of motivated behavior, is common (30% prevalence in de novo PD) and disabling. No specific treatments exist for apathy in PD, likely reflecting poor understanding of its pathophysiology. Goal directed behavior comprises cognitive, affective, and motor components, each of which can be affected in PD. To address this knowledge gap, we implemented in a rat model of PD tasks aimed at: detecting loss of motivation, i.e., discriminating between different components of goal related behavior, and characterizing potential confounders, e.g., motor impairment, hyposmia and anxiety. Male Sprague Dawley rats (N=8) underwent testing before and after bilateral 6-OHDA infusions into dorsolateral striatum (7.5 ug/2uL). PD-like postural stability and akinesia was measured using a forelimb stepping task. Motivation was assessed with an operant food task, i.e., a low-effort (fixed ratio 1) task in which one nose poke resulted in a palatable food reward, and the burrowing task, an ethologically relevant motivation-dependent behavior quantified by measuring the weight of displaced bedding. Olfaction and motor aspects of consumption were measured by determining the times to first contact and complete consumption of palatable food, compared between visually apparent and hidden items. An open field task measured time spent in center vs perimeter zones to indicate anxiety. After lesioning, forelimb stepping decreased ($p<.01$) and distance to first step increased ($p<.01$), validating the PD-like nature of the lesion. There was no change in the open field task, therefore the lesion was not anxiogenic. The burrowing task was unaltered for most rats, indicating that the lesion did not induce generalized

apathy. In the operant food task, the number of active nose pokes decreased during the last three minutes of the session ($p<.05$) after lesioning, but the pellets consumed per minute was stable. There was no change in latency to first poke or number of pellets consumed in the initial response period. This reduction did not reflect hyposmia, as there was no change in the speed of identifying a food pellet with olfactory cues alone. Thus, in a model of early PD, this evidence suggests a motivational deficit that selectively affects effort-based decision making during motivated behavior, and does not appear to involve olfactory dysfunction, nor anxiety, nor an impairment in the ability to perform the tasks. Future investigations will use this model to investigate the neural underpinnings of apathy-like deficits in effort-based decision making.

Disclosures: K.M. King: None. **G.K. McCormack:** None. **J.G. Noel-Torres:** None. **A.F.S. Shack:** None. **A.L. Persons:** None. **T. Napier:** None. **A.E. Kirby:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.07

Topic: C.03. Parkinson's Disease

Support: NSERC Discovery Grant 2020-04302

Title: Acute Effects of Pubertal Microbial Dysbiosis and LPS-Induced Neuroinflammation on Biomarkers Related to Parkinson's Disease

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide following only Alzheimer's disease. Although many theories have attempted to explain the causes of PD, little information exist regarding its etiology. Stressors experienced during puberty can have a profound impact on brain development and functioning. A single exposure to the bacterial endotoxin lipopolysaccharide (LPS) during puberty, in mice, alters the gut microbiota and causes enduring deficits in learning, spatial memory, and sexual receptivity in adulthood. Microbial dysbiosis can influence immune responsivity and make individuals more susceptible to disorders such as, multiple sclerosis, Alzheimer's, and PD later in life (Kennedy et al., 2016). However, the acute effects of pubertal microbial dysbiosis on brain functioning related to PD remains unknown. Thus, the objective of the current exploratory research project is to examine the acute effects of pubertal microbial dysbiosis and LPS treatment on brain functioning related to PD. A total of 56 (28 male and 28 female) CD1 mice were used in this experiment. At five weeks of age, male and female CD1 mice received either broad-spectrum antibiotics or water through gavage, twice a day, for seven days. One week following antibiotic

treatment (6 weeks old), mice received an intraperitoneal injection of either LPS or saline. Sickness behaviors were monitored 2, 4, 6, and 8 hours following the LPS injection. Eight hours following the injection, mice were euthanized and brains were collected. The caudate putamen (CP) and substania nigra (SN) were extracted using micropunches and processed with a real-time quantitative PCR (RT-qPCR) to examine SNCA (synuclein alpha), IL-10, and IL17A mRNA expression. It is expected that antibiotic and LPS treatment will increase SNCA and IL-17A mRNA expression while decreasing IL-10 mRNA expression. The results from this experiment will provide insight on early indicators of PD while also furthering our knowledge of the relationship between the gut-brain axis and the development of the disorder.

Disclosures: P. Esposito: None. **K. Smith:** None. **M. Gandelman:** None. **J. Liang:** None. **N. Ismail:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.08

Topic: C.03. Parkinson's Disease

Support: NIEHS: 5P30 ES019776-08
NIEHS: R01ES029212

Title: Oral insecticide exposure impairs intestinal function and disrupts nigrostriatal dopamine circuitry in adult mice

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Abstract: Pesticide exposure increases the risk of developing Parkinson's disease (PD). PD is a progressive motor neurodegenerative disease attributable to the death of the dopamine (DA) neurons within the nigrostriatal dopamine pathway, resulting in gradual loss of motor function. Sometimes decades before motor symptom onset, individuals with PD experience a range of gastrointestinal (GI) disturbances. Gut dysbiosis also occurs in PD, and growing evidence suggests a contributory role for the gut microbiome in mediating neurological disease states. The emerging associations between PD, pesticide exposure, and GI abnormalities have led to the idea that some cases of PD may be triggered from within the gut after oral pesticide exposure. Pyrethroids, one of the most used insecticides in the world, target the nervous system and disrupt DA signaling pathways, and ingestion is one of the most common routes of pyrethroid exposure. We therefore predicted that low-dose oral exposure to the pyrethroid deltamethrin induces GI

dysfunction and disrupts nigrostriatal DA circuitry in mice. Young and aged wildtype mice of both sexes were orally gavaged weekly with deltamethrin (3 mg/kg) or a corn oil vehicle control using subchronic and chronic exposure paradigms. GI function was assessed by measuring fecal output and total intestinal transit time, and the composition of the gut microbiome was determined by 16S rRNA sequencing. PD-relevant gene and protein expression were quantified in gut and brain tissues via qPCR and western blot. Our results indicate that oral deltamethrin exposure significantly reduces fecal output, indicative of constipation, and alters gut microbial composition. In the brain, oral deltamethrin exposure significantly changed levels of proteins important for DA synthesis and reuptake compared to controls. In support of our hypothesis, these data suggest that low-dose oral pyrethroid exposure induces PD-relevant symptoms in the gut and brain of adult mice. Future studies will investigate specific roles for the gut microbiome and potential routes of transmission along the gut-brain axis in facilitating pyrethroid-induced neurotoxicity.

Disclosures: A.C. White: None. **S. Bandlamudi:** None. **B. Pfluger:** None. **J.R. Chang:** None. **S.D. Kelly:** None. **W. Caudle:** None. **T.R. Sampson:** None.

Poster

P262. Rat and Mouse Toxin and Behavior Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P262.09

Topic: C.03. Parkinson’s Disease

Support: Health and Medical Research Fund 15163051

Title: Lycium barbarum prevents the development of classical motor impairments in the 6-OHDA animal model of Parkinson's disease

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Abstract: Parkinson’s disease (PD) is the second most common neurodegenerative disease worldwide. The disease is mainly characterized by defects in the nigrostriatal pathway, which gives rise to the well-known motor impairments patients experience. As the disease progresses the symptoms worsen, and at late stage PD patients start to experience memory deficits as well. 6-hydroxydopamine (6-OHDA) unilaterally injected into the medial forebrain bundle (MFB) of Sprague-Dawley (SD) rats has been widely used to investigate the development of PD, and this model clearly shows both motor impairments and cognitive deficits. The Chinese medicine

Lycium barbarum (wolfberry) has been shown to modulate microglial functions. Thus, we aimed to investigate whether wolfberry would prevent the development of PD-like symptoms in the classical 6-OHDA animal model. To address this, SD rats were fed either PBS or wolfberry daily prior to stereotaxic injecting sham or 6-OHDA into MFB. Rotarod was used to evaluate gross motor function of the SD rats, where 6-OHDA alone showed a clear reduction in their ability to stay on the rotarod. Interestingly, when SD rats were fed wolfberry prior to their injection of 6-OHDA, no detrimental effects of 6-OHDA on gross motor functions was observed. A similar result was observed when using cylinder test to evaluate fine motor coordination of SD rats. Apomorphine-induced rotation test was used to reflect the induced lesion, and here 6-OHDA injected SD rats showed a complete lesion. Surprisingly, wolfberry did not decrease the net rotations induced by 6-OHDA. Given the promising neuroprotective effects wolfberry had on 6-OHDA-induced motor impairments, we went on to examine spatial learning using Morris Water Maze (MWM). SD rats lesioned with 6-OHDA alone impaired spatial hippocampal functions, whereas feeding wolfberry prior to surgery protected against this. Spontaneous y-maze was used evaluate the spatial working memory. In contrast to the results obtained from MWM, SD rats with a unilateral 6-OHDA lesion showed an increase in spontaneous alternation, thus indicating improved working memory. Together these data clearly reveal a neuroprotective effect of wolfberry on 6-OHDA-induced motor impairment. Future study will focus on investigating how intake of wolfberry elicit the neuroprotective effect in the classical 6-OHDA animal model.

Disclosures: M.H. Soerensen: None. **R.P. Wang:** None. **J.Y. Ho:** None. **R.C. Chang:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.01

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS09295001
NIH F31 NS12069501
T32 NS047987

Title: Comparing the effects of anti-Parkinsonian medication and deep brain stimulation on eye and hand movements during visually-guided reaching

Authors: *M. J. MUNOZ[1], R. ARORA[1], Q. DRANE[1], Y. M. RIVERA[1], G. PAL[2,3], L. VERHAGEN METMAN[2], S. SANI[2], J. ROSENOW[1], L. C. GOELZ[1,4], D. M. CORCOS[1], F. J. DAVID[1];
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Abstract: Anti-Parkinsonian medication and subthalamic nucleus deep brain stimulation (STN DBS), two common treatments of Parkinson's disease (PD), effectively improve upper and lower limb movements. However, there is evidence that these treatments may have differential effects on eye and limb movements. This is interesting because eye and limb movements are both controlled through the basal ganglia loops, but PD and the treatment of PD may affect these effectors differently. Using a task that requires both eye and limb movements, we are able to determine if anti-Parkinsonian medication and STN DBS similarly or differentially affect upper limb and eye movements. Continuing to understand the effects of medication and STN DBS on different aspects of movement may provide insight into how these treatments are differentially affecting neural circuits. We predicted that both medication and STN DBS would improve reaching performance but that medication and STN DBS will have opposing effects on saccade performance. We had participants perform a visually-guided reaching task OFF and ON anti-Parkinsonian medication (n = 36). We also had participants complete the same task OFF and ON bilateral STN DBS while OFF medication (n = 16). Participants were instructed to fixate on a central point until a peripheral target appeared, which was the cue to then look and reach to the target. Saccade and reaching peak velocity and latency were determined using a custom MATLAB script and statistical analyses were performed in SAS using mixed models. We found that anti-Parkinsonian medication significantly decreased saccade peak velocity, while increasing reaching peak velocity. Medication increased saccade latency while having no effect on reaching latency. Additionally, bilateral STN DBS significantly increased saccade and reaching peak velocity, and decreased saccade latency while having no effect on reaching latency. These findings suggest that medication and STN DBS have differential effects on eye movements. Medication reduces saccade velocity and increases latency, while STN DBS increases saccade velocity and reduces latency. Conversely, medication and STN DBS have similar effects on visually-guided reaching, in which medication and STN DBS improve peak velocity while having no effect on latency.

Disclosures: M.J. Munoz: None. **R. Arora:** None. **Q. Drane:** None. **Y.M. Rivera:** None. **G. Pal:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, Bakalar Family Foundation, Parkinson's Foundation. F. Consulting Fees (e.g., advisory boards); Asim, Huron, AbbVie, Boston Scientific, KeyQuest, Qessential, SeaGrove, Medtronic, Abbott. **L. Verhagen Metman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, Abbott, AbbVie, Boston Scientific, Biogen, Medtronic, Neuroderm, Prilenia Therapeutics. F. Consulting Fees (e.g., advisory boards); Abbott, AbbVie, Boston Scientific, Biogen. **S. Sani:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, Medtronic, Abbott, Boston Scientific. **J. Rosenow:** F. Consulting Fees (e.g., advisory boards); Boston Scientific. **L.C. Goelz:** None. **D.M. Corcos:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report

that research relationship even if those funds come to an institution.; NIH, Michael J Fox Foundation. **F.J. David:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.02

Topic: C.03. Parkinson's Disease

Support: Swiss National Science Foundation PZ00P3_186142
Swiss Parkinson Foundation

Title: Stimulation maps to guide programming of deep brain stimulation

Authors: *T. NGUYEN[1,2], A. SEGURA AMIL[1,2], S. BARLATEY[1], D. ZHANG[1], M. MONTALBETTI[1], A. NOWACKI[1], C. POLLO[1];
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Abstract: Deep brain stimulation is an established treatment for movement disorders such as advanced Parkinson's disease and essential tremor. The new generation of segmented leads provide clinicians with unprecedented possibilities to steer the electrical stimulation towards regions with high probability of therapeutic effect and away from regions with high probability of side effects. This holds considerable promise to improve patient outcome. However, these new leads have rendered stimulation programming more difficult and both clinicians and patients would benefit from programming tools.
Stimulation maps can provide a visual guide and highlight effective regions of stimulation. In future these maps can be used with *computer-assisted programming* to recommend stimulation settings. We have computed two stimulation maps: one map for the subthalamic nucleus to help treat Parkinson's disease, another map for the ventral intermediate nucleus of the thalamus and the posterior subthalamic area to help treat essential tremor.
For the Parkinson's disease map, we have retrospectively analyzed clinical mapping data from 83 implanted patients from two centers. First, the patients' leads were reconstructed with the open-source toolbox Lead-DBS in Matlab. Second, the volumes of tissue activated were estimated also within Lead-DBS. These volumes were weighted by their clinical score and aggregated in template space for the stimulation map. Third, voxel-wise statistical testing identified a region with significantly better than average therapeutic effect. This 'sweet spot' was inside the dorsolateral portion of the subthalamic nucleus. Forth, the overlap ratio of a stimulation volume with the sweet spot was predictive of outcome and could be used in future

for computer-assisted programming pending further studies.
Similarly for the essential tremor map, we have retrospectively analyzed chronic stimulation data from 70 patients from four centers. The sweet spot of this map hinted at the dentato-rubro-thalamic tract as the underlying structure of therapeutic effect. But a more refined analysis will be needed for prediction and computer-assisted programming.

Disclosures: T. Nguyen: None. **A. Segura Amil:** None. **S. Barlatey:** None. **D. Zhang:** None. **M. Montalbetti:** None. **A. Nowacki:** None. **C. Pollo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of Aleva Neurotherapeutics SA.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.03

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R15NS098342

Title: The influence of long-term transcranial direct current stimulation on motor learning in Parkinson's disease

Authors: *M. PANTOVIC[1], N. PUDAR[1], L. L. ALBUQUERQUE[1], S. BROEDER[2], D. MORRIS[1], E. WILKINS[1], M. R. LANDERS[1], Z. MARI[3], B. POSTON[1];
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Abstract: Transcranial direct current stimulation (tDCS) applied to the primary motor cortex (M1) improves acute motor skill acquisition in upper limb tasks in Parkinson’s disease (PD). However, the magnitude to which tDCS may be able to improve long-term motor learning in PD has not been established. The purpose was to determine the influence of long-term application of tDCS on motor learning in PD. The study utilized a randomized, double-blind, SHAM-controlled, between-subjects experimental design. Eleven men and women with PD were allocated to either a tDCS group (n = 6) or a SHAM stimulation group (n = 5). Each participant performed 9 practice sessions over a 2-week period that involved extensive practice of a complex isometric precision grip task (PGT). In addition, participants performed the PGT in 4 testing sessions that occurred at baseline and at 1, 14, and 28 days after practice ended (EOP+1, EOP+14, and EOP+28). The practice sessions were performed simultaneously with either anodal tDCS or SHAM stimulation applied over the hand area of the M1 contralateral to the primarily affected hand. tDCS was delivered at a current strength of 1 mA for a duration of 25 minutes using a M1-supraorbital electrode montage. For SHAM stimulation, the current was ramped up

and down over a 60 second period according to established blinding procedures. The PGT task involved accurately matching a target sine wave template on a computer monitor by controlling the sum of the index finger and thumb forces. All practice and test sessions were performed while participants were on medications. The primary outcome measure was the force error in the PGT, which was quantified as the average error in force relative to a target force template in each experimental session. Force error was analyzed with a 2 *Group* (tDCS, SHAM) x 4 *Test* (Baseline, EOP+1, EOP+14, EOT+28) ANOVA. The *Group* x *Test* interaction ($P = 0.771$) and *Group* main effect ($P = 0.449$) were not significant. There was a significant main effect ($P < 0.001$) for *Test* and post hoc analysis indicated that force error was lower for EOT+1, EOT+14, and EOT+28 compared to Baseline (all Ps < 0.002). However, the reduction in force error from Baseline to the 3 testing sessions was 8%, 15%, and 19% greater in the tDCS group, respectively. Although not significant due to the current low sample size and inter-individual variability, the findings indicate that long-term application of tDCS may improve motor learning to slightly greater extent than practice alone in PD.

Disclosures: M. Pantovic: None. **N. Pudar:** None. **L.L. Albuquerque:** None. **S. Broeder:** None. **D. Morris:** None. **E. Wilkins:** None. **M.R. Landers:** None. **Z. Mari:** None. **B. Poston:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.04

Topic: C.03. Parkinson’s Disease

Support: Swiss National Science Foundation PZ00P3_186142
Swiss Parkinson Foundation

Title: Patient-specific hyperdirect pathway activation in DBs for Parkinson's disease

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Abstract: Deep Brain Stimulation (DBS) is an effective treatment in advanced Parkinson’s disease patients to control motor symptoms such as bradykinesia, rigidity, or tremor. DBS leads are implanted in the basal ganglia, which is connected to the cortex through, e.g., the direct, indirect and hyperdirect pathways. Recent studies suggest that DBS stimulation of the hyperdirect pathway plays an important role in the effects of the treatment.
The aim of the study was to determine an activation threshold of the hyperdirect pathway that results in the control of motor symptoms while avoiding the appearance of side effects due to activation of the corticospinal tract.

The study included 20 Parkinson's disease subjects (9 female) undergoing bilateral DBS of the subthalamic nucleus. Diffusion weighted MRI was used to perform whole-brain tractography in MRtrix3. Data pre-procesing included denoising, Gibbs unringing and distortion and eddy-current correction. The filtering algorithm SIFT2 was applied to the whole-brain tractogram to assign weights to the generated streamlines. Lead reconstruction was performed in the Lead-DBS toolbox (v2.2). Patients were clinically assessed six months after surgery, and the stimulation settings that resulted in clinical effect and appearance of side effects were used to estimate volumes of tissue activated. The hyperdirect pathway and corticospinal tract were extracted from the whole-brain tractogram using cortical and subcortical regions of interest and regions of avoidance. Finally, activation thresholds for the hyperdirect pathway and corticospinal tract were obtained using a logistic regression model.
We could establish an activation threshold of 60% of the hyperdirect pathway weights to obtain a full clinical effect, and an activation threshold of 3% of the corticospinal tract weights for the appearance of side effects.
An accurate activation threshold of the hyperdirect pathway will allow in the future a more informed and precise targeting of the hyperdirect pathway in DBS treatments for Parkinson's disease to control motor symptoms and avoid the appearance of side effects.

Disclosures: A. Segura Amil: None. **T. Nguyen:** None. **A. Nowacki:** None. **C. Pollo:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.05

Topic: C.03. Parkinson's Disease

Support: University of Colorado Training Grant

Title: Naturalistic sleep in Parkinson's disease assessed by actigraphy and direct recordings of the subthalamic nucleus

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Abstract: Sleep disturbances are highly prevalent in patients with Parkinson's Disease (PD), and are closely linked with cognition, mood disorders, and patient-reported quality of life. Although deep brain stimulation (DBS) is primarily used to treat PD motor symptoms, several studies have shown added benefit for non-motor symptoms including sleep disturbance. Prior studies have used DBS electrodes to record subthalamic nucleus (STN) oscillatory activity in the form of local field potentials (LFPs) during sleep cycles. In PD patients, unique spectral patterns of STN

activity between the awake, rapid eye movement (REM) sleep, and non-REM (NREM) sleep states have been reported and provide a novel insight into sleep architecture and basal ganglia physiology. Interpretation of these findings has been technologically limited, as recordings can only be obtained during a single night inpatient hospitalization with the patient physically attached from the cranium to external equipment. In this study, we utilized a recently FDA-approved implanted pulse generator (IPG), which allows for recording LFPs simultaneously with the delivery of therapeutic stimulation, to assess sleep in the patient's home environment. Sleep was assessed by daily self-report and wrist-based actigraphy. In n = 5 subjects, recorded for 14 days/nights, we observed that LFP power in the beta frequency range (13 - 30 Hz) increases by a factor of two in the overnight hours compared to daytime, which is correlated with patient sleep times. These results provide proof of concept that an electrophysiologic biomarker recorded from the STN, using standard-of-care DBS equipment, can reliably assess sleep in the home setting. The experimental design allows for a substantial increase in the amount of data that can be collected from an individual patient and facilitates studying between-night differences in sleep architecture, which was previously not possible. Improved understanding of sleep architecture may lead to novel approaches to improve sleep disturbances in this population.

Disclosures: A.J. Baumgartner: 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.; University of Colorado Department of Neurology. **L. Hirt:** None. **D.S. Kern:** 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, Medtronic, Parkinson's Foundation, University of Colorado Department of Neurology. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Boston Scientific, AbbVie Pharmaceutics. F. Consulting Fees (e.g., advisory boards); Colorado Clinical and Translational Sciences Institute (CCTSI) Data Safety Monitoring Board, Boston Scientific, AbbVie Pharmaceutics. **J.A. Thompson:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Medtronic, Boston Scientific.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.06

Topic: C.03. Parkinson's Disease

Support: NIH R37 NS040894

Duke Compute Cluster

Title: Analyses of local field potentials recorded from deep brain stimulation electrodes in thalamus

Authors: *K. KUMARAVELU[1], S. L. SCHMIDT[1], Y. ZHAO[1], B. D. SWAN[1], C. S. OZA[1], J. J. PETERS[1], D. A. TURNER[2], W. M. GRILL[1];
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Abstract: Deep brain stimulation (DBS) of the ventral intermediate nucleus (VIM) of the thalamus (TH) is a surgical intervention that is effective in managing the symptoms of tremor in essential tremor (ET) and tremor dominant Parkinson's disease (PD). Despite the widespread use of VIM-DBS, its mechanism of action remains unclear. Further, most current DBS systems are open-loop, i.e., stimulate continuously. One of the key challenges to developing closed-loop DBS is identifying neural biomarkers that correlate with tremor. We recorded local field potentials (LFPs) from the TH using a bipolar pair of the four contact DBS lead (Model 3387 and 3389, Medtronic) in ET and PD patients who underwent surgery for either lead implantation or pulse generator replacement (n=33). Contact location was determined by identifying known anatomical landmarks (anterior commissure (AC)-posterior commissure (PC) coordinates) in pre-and post-operative MRI and CT images. We simultaneously recorded tremor using a triaxial accelerometer (Accel) placed on the hand dorsum. The spectral power of the Accel data was used as a measure of tremor. Tremor was analyzed with respect to the spectral power of LFP data and the magnitude square coherence between Accel and LFP time-series data. Finally, we used a computational model of VIM, ventral caudalis (Vc) and ventral oralis anterior/posterior (Voa, Vop) regions of the TH to determine the biophysical basis of the recorded LFP signals. The thalamic model was populated with a proportion of thalamocortical relay cells firing in burst, random, regular modes, and the distribution of burst cells across the TH was based on microelectrode recordings from humans. DBS contact locations were spread across the TH, with most lying within the Vop and VIM regions (mean distance anterior to PC: 6.4 mm, mean distance above AC-PC line: 3.8 mm). There was no correlation between tremor and LFP spectral power in the tremor band (rho = 0.0505, P = 0.36). However, there was a strong correlation between tremor and peak coherence between LFP and Accel data across all axes (rho > 0.6, P < 0.01). The relationship between peak Accel frequency and peak coherence frequency was linear with a slope of ~1 for most patients. However, few patients had peak coherence frequency double that of Accel frequency (n=9/33). Finally, the spectral power of the simulated LFP in the tremor band varied considerably with electrode locations across TH regions. This might explain the differences in LFP spectral power and lack of correlation with tremor seen in the experimental data. Understanding the biophysical basis of LFPs recorded from DBS electrode enables better identification of robust biomarkers for tremor.

Disclosures: K. Kumaravelu: None. **S.L. Schmidt:** None. **Y. Zhao:** None. **B.D. Swan:** None. **C.S. Oza:** None. **J.J. Peters:** None. **D.A. Turner:** None. **W.M. Grill:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.07

Topic: C.03. Parkinson's Disease

Support: MJFF Therapeutic Pipeline Program

Title: Cortical and Subcortical Gait Related Neuronal Modulation During Free Walking in Parkinson's Disease Patients

Authors: *K. H. LOUIE, M. S. YAROSHINSKY, P. A. STARR, D. D. WANG;
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Abstract: Cortical and Subcortical Gait Related Neuronal Modulation During Free Walking Parkinson's Disease Patients[1]Kenneth H. Louie PhD, [1]Maria S. Yaroshinsky BS, [1]Philip A. Starr, MD, PhD, [1]Doris D. Wang MD, PhD
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Gait impairment in Parkinson's disease (PD) is a particularly debilitating symptom and refractory to conventional treatment. The initial success of adaptive stimulation paradigms in treating akinesia in PD has garnered much interest for its use to improve gait in PD. However, in order to apply adaptive stimulation algorithms for gait, gait-related biomarkers are needed. While few studies have reported gait-dependent changes in cortical or basal ganglia local field potentials (LFPs), simultaneous cortical and subcortical LFPs dynamics in relation to the gait cycle have not been studied. Here, we record sensorimotor cortical and basal ganglia LFPs from PD patients during natural, overground walking using a totally implanted bidirectional neurostimulator, Summit RC+S (Medtronic).Four PD individuals consented to participate in this study. Three individuals underwent implantation of bilateral subdural cortical paddles overlying the primary motor (M1) and sensory (S1) cortices, with deep brain stimulation (DBS) leads targeting either the bilateral subthalamic nuclei (STN) or globus pallidus interna (GPi). One individual was implanted unilaterally on the left side, with the same cortical paddle location and a DBS lead in the GPi. Brain electrodes are connected to the bilateral RC+S devices. LFPs were recorded in the on-medication, off-DBS state. Gait kinematics were measured using inertial external sensors systems, Delsys Trigno and Xsens Awinda, while the patients performed overground walking. LFP time frequency and wavelet analysis was performed across all electrodes and synchronized to each participants gait cycle. We found cyclical, gait-event and gait-cycle modulated LFP power changes in the theta (4-7 Hz), alpha (8-12 Hz), and beta (13-30 Hz) band frequencies. Increases in beta was mainly associated with heel-strike and toe-off gait events in subcortical and M1, while theta and alpha increases were mainly associated with heel-strikes only in S1. Theta, alpha, and beta band gait-related modulations was found in all recorded areas, but not in all participants. These results show that there is high modulation of motor cortical and STN/GPi low frequency power during the natural gait cycle in PD patients. These

dynamic network changes have the potential to be used as control signals for adaptive DBS synchronized to the gait cycle.

Disclosures: K.H. Louie: None. **M.S. Yaroshinsky:** None. **P.A. Starr:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US patent 9,295,838. **D.D. Wang:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.08

Topic: C.03. Parkinson's Disease

Support: VA Merit Grant RX001613
Sheffield Memorial Grant PSG00037

Title: Daily Low-dose Niacin Supplementation Modulates Symptoms, Inflammation, andα-synuclein in Parkinson's Disease Subjects

Authors: *M. SEAMON[1,2], S. PUROHIT[1,2], B. GIRI[3], R. CHONG[1,2], J. C. MORGAN[4,2], C. WAKADE[2,1];
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Abstract: Background: Parkinson's disease (PD) patients have lower levels of niacin compared to their age-matched, healthy counterparts. We conducted a clinical trial to restore levels of niacin in PD patients by supplementing 250 mg q.d. in addition to the subjects' typical medications. Forty-seven patients were recruited in the double-blind, placebo-controlled trial for six months (6MO), followed by 6MO of open-label niacin. All participants gave written informed consent prior to any study procedures. The randomization code was not broken until all subjects had completed the two phases of the trial. **Outcomes:** Motor symptoms and plasma levels of serotonin, inflammatory mediators, and α-synuclein were evaluated at each visit. **Methods:** Motor symptom improvement was measured using the motor portion (part III) of the Unified Parkinsons Disease Rating Scale (UPDRS). Serotonin levels were measured by HPLC. Inflammatory mediators and α-synuclein were evaluated by immunoassays. **Results:** During open-label niacin treatment (6MO-12MO), UPDRS III scores significantly decreased by 4.63 points in the niacin group (12MO of niacin), but there was no significant change in the placebo group (6MO of niacin).Serotonin levels significantly decreased by an average of 25.34 mg/dL after 6MO of placebo (p=0.011), while the niacin group had no change.In the first 6MO of the trial, the placebo group showed a significant reduction in the plasma levels of MIP-1β (p=0.012). A decrease in IP-10 (p=0.0009) was observed at 12MO compared to baseline in the

placebo group. The niacin group had significantly increased MIP-1α (p=0.02) levels at 6MO compared to baseline. Plasma levels of total α-synuclein were not different between groups with a 40% average change over six months. The mean level of α-synuclein oligomers decreased in both groups but only reached significance with the first six months of niacin treatment (p=0.048). The average level of p(ser129) α-synuclein was significantly decreased in the niacin group over 12MO (p=0.010) but increased in the placebo group (p=0.012). **Conclusion:** Together, these results suggest a disease-modifying effect of niacin in PD patients, unlike Sinemet and other common PD medications, which only aim to reduce bothersome motor and non-motor symptoms. Future clinical trials will determine whether niacin is truly a disease-modifying vitamin and should be an adjunct therapy alongside typical PD medications.

Disclosures: M. Seamon: None. **S. Purohit:** None. **B. Giri:** None. **R. Chong:** None. **J.C. Morgan:** None. **C. Wakade:** None.

Poster

P269. Parkinson's Disease: Therapeutic Strategies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P269.09

Topic: C.03. Parkinson’s Disease

Title: Strain-level meta-analysis of Parkinson’s disease-associated gut microbiome

Authors: *K. WEST, E. RUTHERFORD, N. NARAYAN, S. IWAI, J. CHOI, S. VISWANATHAM, P. LAL, T. Z. DESANTIS;
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Abstract: Parkinson’s disease (PD) is a progressive neurological disorder that can be initiated by pathogenic protein-protein interactions (PPI) in the gastrointestinal (GI) tract in murine models. Namely, α-synuclein, a soluble presynaptic neuronal protein, forms aberrant aggregations in PD patients. Introduction of α-synuclein aggregates into the mouse GI tract induces α-synuclein aggregates in the brain via vagal propagation. In an α-synuclein overexpression model, aggregation was found to be dependent on GI microbial activity and, furthermore, a bacterial chaperonin protein has been shown to prevent aggregation of α-synuclein in vitro. Thus, we sought to identify from the literature the gastrointestinal bacterial strains depleted in PD as the first step in high-throughput discovery of the diverse bacterial proteins with potential therapeutic PPI with α-synuclein to reduce aggregation. Unfortunately, past reports of microbiome associations with PD have two limitations: 1) results are not concordant across cohorts and 2) the data is not resolved to specific strains. We overcame these challenges and identified precise bacterial strains associated with PD in multiple cohorts. By harmonizing publicly available microbiome sequencing data and annotating it against the StrainSelect database, we were able to conduct the first strain-level, and largest meta-analysis of PD to date. Raw 16S rRNA gene

amplicon or metagenomic sequencing data were collected from 8 studies across 5 countries and included a total of 992 PD patients and 663 control subjects. Control cohorts consisted of subjects without neurodegenerative disease. Raw data was processed using standardized pipelines to measure strain abundances. Effect sizes between case and control patients for each strain were calculated and random effects models were used to estimate the concordance of effect sizes across studies. In total, 168 and 441 bacterial strains were significantly (FDR<0.2) depleted or enriched in PD patients compared to controls, respectively. Specific known previously-isolated microbial strains from the StrainSelect database were identified with statistical support (FDR<0.2) as either elevated in PD, such as strains of Lachnospira eligens and Bacteroides_B vulgatus, or diminished in PD patients such as strains of Prevotella and Bacteroides_A plebeius. Our unique approach and findings enable therapeutic development since specific strains, and their expressed proteins and metabolites, can now be screened for interactions with target proteins of the central nervous system.

Disclosures: K. West: None. **E. Rutherford:** None. **N. Narayan:** None. **S. Iwai:** None. **J. Choi:** None. **S. Viswanatham:** None. **P. Lal:** None. **T.Z. DeSantis:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.01

Topic: C.03. Parkinson’s Disease

Support: 5UG3NS105703-02

Title: A Parkinson’s Disease-Chip model system to study early onset sporadic Parkinson’s disease.

Authors: *M. OTERO[1], A. LAPERLE[1], S. SANCES[2], W. SPIVIA[3], S. BELL[1], D. LALL[1], B. ONDATJE[1], J. VAN EYK[3], C. N. SVENDSEN[1];
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Abstract: The molecular and cellular changes in midbrain cells that underlie Parkinson’s disease (PD) are complex and likely involve the interplay and eventual failure of multiple cellular systems. While traditional culture models show promise for identifying such underlying mechanisms, these monoculture models likely lack the cellular complexity and developmental maturity to capture complex changes underlying PD. Previous work from our group with microphysiological organ-chips demonstrated improved neuronal maturity when co-cultured with multiple cell types including brain microvasculature endothelial cells and astrocytes and significant changes in synuclein levels in iPSC derived dopamine neurons from patients with

early onset PD. The current objective is to develop robust and reproducible human vascularized models of midbrain tissue as a PD-chip, determine cellular and molecular disease signatures, and test the efficacy and blood-brain barrier transport of candidate therapeutics. iPSC derived dopamine neurons can survive in the top channel of the chips for up to 28 days along with isogenic brain derived endothelial cells on the bottom of the chip creating a functional blood brain barrier. Comprehensive cellular and molecular studies are ongoing, including genomic, transcriptomic, proteomic, metabolomic, and electrophysiological assays. Single nuclei RNA-sequencing demonstrates multiple cell types within these PD-chips. With this model in place we are now testing various approaches to modulating synuclein levels including the use of oligonucleotides that can be administered either to the brain side or the blood side of the 2-chamber PD chip in order to simulate the ability of the compound to cross the blood-brain barrier and assess any reversal of disease signature.

Disclosures: M. Otero: None. **A. Laperle:** None. **S. Sances:** None. **W. Spivia:** None. **S. Bell:** None. **D. Lall:** None. **B. Ondatje:** None. **J. Van Eyk:** None. **C.N. Svendsen:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.02

Topic: C.03. Parkinson’s Disease

Title: Altered HSPA5 proteostasis may be a key disease phenotype in young onset sporadic Parkinson’s Disease

Authors: *A. LAPERLE[1], M. G. OTERO[1], N. YUCER[1], A. STOTLAND[2], J. VAN EYK[3], C. N. SVENDSEN[1];
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Abstract: Parkinson’s Disease (PD) is classically characterized by the progressive loss of dopamine (DA) neurons in the substantia nigra as well as the presence of cytoplasmic inclusions known as Lewy bodies and Lewy neurites within specific brain regions. Inclusions contain large amounts of the protein α-synuclein, which is also associated with a rare form of genetic PD caused by triplication of the *SNCA* gene. iPSC-based models derived from patients with monogenic mutations in PD-related genes show accumulation of α-synuclein in DA neurons; however, monogenic mutations account for only a minority of PD cases. Our publication showed that induced pluripotent stem cells (iPSCs) from sporadic young onset PD (YOPD) patients have a distinct disease signature that included an accumulation of α-synuclein, as well as other disease-related phenotypes including reduced lysosomal proteins and function. We found that a class of phorbol ester compounds can correct this disease signature. We are now investigating

the molecular alterations that cause this disease signature and the efficacy of phorbol ester drugs to alter these phenotypes. In order to study complex protein dynamics in these DA neuron cultures, we employed a novel pulse-chase azidohomoalanine (AHA) labeling technique. The AHA label is incorporated into newly synthesized proteins during the pulse and degradation of newly synthesized proteins is monitored through a subsequent chase. Analysis of DA using this AHA paradigm demonstrates significantly altered synthesis and degradation of labeled proteins in YOPD iPSCs compared to control iPSCs. Critically, YOPD neurons exhibited reduced synthesis of the key endoplasmic reticulum (ER) chaperone and ER-associated degradation (ERAD) master regulator BIP/HSPA5 as well as reduced turnover of this important protein. High resolution imaging of these YOPD neurons indicated α-synuclein pathology in perinuclear/ER regions. Interestingly, the phorbol esters identified in our previous work that stimulate proteasomal dependent α-synuclein degradation also led to not only dephosphorylation of BIP/HSPA5 but also increased protein levels. The impaired synthesis of BIP/HSPA5 and perinuclear α-synuclein accumulation in YOPD and the responsiveness of this key regulator to a class of compounds that alter the distinct disease signature identified in these neurons suggest that ERAD is a critical pathway in the molecular pathogenesis of YOPD.

Disclosures: A. Laperle: None. **M.G. Otero:** None. **N. Yucer:** None. **A. Stotland:** None. **J. Van Eyk:** None. **C.N. Svendsen:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.03

Topic: C.03. Parkinson’s Disease

Support: Paul G. Allen Frontiers Group Grant 12879

Title: Combining human iPSC and Organ-Chip technologies for modeling the gut-brain axis in patients with Parkinson’s disease

Authors: *M. WORKMAN, J. MOSKOWITZ, D. NABOULSI, A. BREDA, D. P. B. MCGOVERN, S. DEVKOTA, C. N. SVENDSEN;
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Abstract: Interaction between the gut microbiome and central nervous system (CNS) has been implicated in a range of neurodegenerative conditions including Parkinson’s disease (PD). However, the connection between the microbiome, gut, and brain has been particularly difficult to study in humans and animal models. Using induced pluripotent stem cell (iPSC) technology, patient-specific cells can be differentiated into cell types relevant to PD. Poly(dimethylsiloxane)-based microphysiological systems, also known as Organ-Chips, have microengineered 3D

chambers that enable the co-culture of different iPSC-derived cell types to model conditions of human disease *in vitro.* Here we present our progress in developing an Intestine-Chip and Brain-Chip from iPSCs that recapitulate several *in vivo* molecular functions and can be fluidically linked to simulate the gut-brain axis. The top channel of the Intestine-chip contains barrier forming intestinal epithelial cells, which when combined with continuous media flow enables co-culture with complex intestinal microbiota. The Brain-Chip contains a top channel seeded with disease-relevant dopaminergic neurons and microglia, and a bottom channel lined by brain microvascular endothelial-like cells. The porous membrane between the two channels allows for the establishment of a blood-brain barrier. We are now using this multi-organ-chip platform to interrogate the contribution of various gut microbes and microbial metabolites in promoting CNS disease pathology. By generating these from patients with heritable forms of PD, we are beginning to explore how systemic organ-organ interactions and microbial crosstalk are involved in promoting neurodegenerative disease.

Disclosures: M. Workman: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents related to chip technology. **J. Moskowitz:** None. **D. Naboulsi:** None. **A. Breda:** None. **D.P.B. McGovern:** None. **S. Devkota:** None. **C.N. Svendsen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents related to chip technology.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.04

Topic: C.03. Parkinson’s Disease

Support: FNR Core Junior Grant CD19/BM/13535609

Title: Miro1 mutant midbrain organoids show mitochondrial dysfunction and dopaminergic neuron loss

Authors: *C. SARAIVA[1], G. SACRIPANTI[1], A. CHEMLA[1], G. ARENA[1], R. KRÜGER[1,2,3], J. C. SCHWAMBORN[1];
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Abstract: Parkinson’s disease (PD), a multifactorial and complex disorder, affects 1 to 2% of the population over 65 years of age and its incidence is rising. PD is mainly characterized by the selective degeneration of dopaminergic neurons in the nigro-striatal pathway. The low levels of

dopaminergic fibers observed in the striatum of patients at PD onset support the hypothesis that axons die back prior to neuronal perikaryal degeneration. Dysregulation of pathways associated with mitochondrial function is one of the mechanisms that might explain this phenomenon. Interestingly, the protein Miro1 (encoded by RhoT1), an element of the motor/adaptor complex, has a key role in mitochondrial trafficking, morphology and calcium-buffering capacity. Miro1 also interacts with several PD related genes including Parkin, Pink1, LRRK2 and α-synuclein. In fact, an altered function of Miro1 was found in fibroblast of PD patients. Finally, in 2019, mutations in the RhoT1 gene were for the first time reported as causative of PD.Here, we investigate the role of Miro1 in an eventual accumulation of dysfunctional mitochondria in the axons of dopaminergic neurons, which might result in retrograde neurodegeneration. To assess this, we used induced pluripotent stem cells obtained from a PD patient with a RhoT1 mutation (p. R272Q) to generate midbrain organoids (MO). The MO we developed resemble the human midbrain in terms of structural organization and cellular composition. MO are enriched in dopaminergic neurons (~60% at day 30) and recapitulate key pathological features of PD. Miro1 mutant MO were assessed at different time points: 20, 30, 60 and 90 days in culture. qPCR analysis showed no alteration in RhoT1 mRNA levels compared with healthy controls. High content imaging analysis and western blotting showed a significant reduction in the levels of tyrosine hydroxylase (TH, dopaminergic neuron marker) in mutants MO from day 30 onwards as well as a lower TH complexity than healthy MO. Mitochondrial impairments were observed in Miro1 mutant MO using the Agilent Seahorse cell mito stress test at day 35. Interestingly, at the same time point, levels of mitochondrial specific reactive oxygen species, measured by MitoSOX-based flow cytometry, are increased in Miro1 mutant MO. Altogether, our work current findings not only further corroborate the utility of our MO model for PD studies, but it also supports the involvement of Miro1 in mitochondrial impairments. Therefore, we provide data that elucidate the role of Miro1 in PD, which might point for a PD dopaminergic degeneration caused by Miro1-dependent mitochondrial dysfunction.

Disclosures: C. Saraiva: None. **G. Sacripanti:** None. **A. Chemla:** None. **G. Arena:** None. **R. krüger:** None. **J.C. Schwamborn:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.05

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS105783
NIH 3R01NS105783-04S1

Title: Role of Brain Derived Neurotrophic Factor in Regulating Mitochondrial Function and Dendrite Homeostasis

Authors: *M. SWAIN[1], R. DAGDA[2], M. DAGDA[2], R. K. DAGDA[2];
[1]Univ. of Nevada, Reno Integrative Neurosci. Grad. Program, Reno, NV; [2]Pharmacol., Univ. of Nevada, Reno, NV

Abstract: **Background:** Mutations in PTEN-induced Kinase 1 (PINK1), a large serine/threonine kinase targeted to mitochondria, leads to juvenile onset forms of Parkinson's Disease (PD). We have recently published that PINK1-deficient primary cortical neurons exhibit a significant loss of dendritic arbors characterized by fragmented mitochondria, increased oxidative stress, and reduction in dendritic mitochondria. In addition, PINK1-deficient neurons show a reduction in the synthesis and extracellular release of mature form of BDNF, a neurotrophin that promotes dendrite outgrowth by binding to the tropomyosin receptor kinase B (TrkB). In addition, previous studies have shown that a cleaved form of BDNF can modulate oxidative phosphorylation in mitochondria suggesting that BDNF can confer neuroprotection in part by maintaining mitochondrial health. **Problem:** the molecular mechanisms by which the mature form of BDNF modulates mitochondrial function remains to be elucidated. We hypothesize that BDNF modulates mitochondrial function, morphology, and content in dendrites to maintain dendritic arbors in healthy neurons and reverse dendrite/mitochondrial pathology in PINK1-deficient neurons. **Methods/Results:** preliminary data obtained from time-lapse confocal microscopy, immunofluorescence-based assays, and Seahorse Bioscience XF24e Analyzer, shows that exogenous administration of human recombinant BDNF (35 ng, 2-24 hrs) can reverse mitochondrial fragmentation by stimulating PKA-mediated phosphorylation of Drp1, restores anterograde mitochondrial trafficking in dendrites, enhances oxidative phosphorylation and transmembrane potential, and restores mitochondrial trafficking in PINK1-deficient primary cortical neurons similar to wild-type. In an *in vitro* chemical model of PD, we observed that exogenous administration of BDNF reversed the loss of dendrites and blocked cell death induced by an acute exposure to the complex I inhibitor rotenone. Mechanistically, time lapse confocal imaging data suggest that BDNF regulates mitochondrial function in part by fostering the trafficking of TrkB-BDNF signaling endosomes to the mitochondrion. In aggregate, our compiled fluorescence and biochemical data suggest that BDNF regulates mitochondrial health, morphology, and function to maintain dendrite health in healthy neurons and confer neuroprotection against ROS-mediated cell death in two *in vitro* genetic and chemical models of PD.

Disclosures: M. Swain: None. **R. Dagda:** None. **M. Dagda:** None. **R.K. Dagda:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.06

Topic: C.03. Parkinson's Disease

Support: Michael J Fox foundation
JPB Foundation

Title: Genetic disruption of mitochondrial complex I triggers progressive, levodopa-responsive Parkinsonism with nigral determinants

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Abstract: Loss of mitochondrial complex I (MCI) in substantia nigra (SN) dopaminergic neurons is a hallmark of Parkinson's disease (PD). Yet, whether this change contributes to PD pathogenesis is unclear. To address this question, intersectional genetics was used to delete the catalytic subunit of MCI in mouse dopaminergic neurons. Surprisingly, MCI disruption induced a Warburg-like shift in metabolism that enabled neuronal survival, but which triggered a progressive loss of the dopaminergic phenotype that was first evident in nigrostriatal axons. This axonal deficit was accompanied by motor learning and fine motor deficits, but not by frank levodopa-responsive Parkinsonism - which only emerged with later loss of SN dopamine (DA) release. Thus, MCI dysfunction alone is sufficient to cause a progressive, human-like Parkinsonism in which loss of nigral DA release makes a critical contribution to motor dysfunction - contrary to the current PD paradigm.

Disclosures: P. Gonzalez Rodriguez: None. **E. Zampese:** None. **K. Stout:** None. **J.N. Guzman:** None. **E. Ilijic:** None. **B. Yang:** None. **T. Tkatch:** None. **L. Gao:** None. **M.A. Stavarache:** None. **M.G. Kaplitt:** None. **J. López-Barneo:** None. **P. Schumacker:** None. **D. Surmeier:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.07

Topic: C.03. Parkinson's Disease

Support: APDA Post-Doctoral Fellowship

Title: The gut microbiome influences mitochondrial function and motor symptoms in α-synuclein overexpressing mice

Authors: *L. H. MORAIS[1], L. STILES[2], M. LADINSKI[1], J. BOKTOR[1], S. MAHMOUDIANDEHKORDI[3], J. JONES[1], R. TSE[4], F. GAO[1], T.-F. CHOU[1], J. TRINH[4], R. KADDURAH-DAOUK[5], S. K. MAZMANIAN[1];
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Abstract: Gut microbiome-brain interactions have been implicated in a wide range of neurological conditions, including Parkinson's disease (PD). Motor dysfunction in PD is primarily associated with the selective dysfunction and loss of nigrostriatal dopaminergic neurons, potentially due to their relatively high energetic demand in comparison to other neurons. In this context, defects of mitochondria function may underlie vulnerability to neurodegeneration through effects on cellular respiration and accumulation of oxygen reactive species. Our group has recently demonstrated that the gut microbiome mediates motor deficits, induces neuroinflammation, and exacerbates α-synuclein (α-syn) pathology in mice. Various metabolites produced by gut bacteria have the potential to modulate host metabolism, but a link between the microbiome and brain mitochondrial function remains to be determined. Using α-syn overexpressing (ASO), we investigated the influence of the microbiome on mitochondrial function and motor performance. Herein, we reveal that the gut microbiome induces accumulation of α-syn in mitochondria from the mouse brain, and alters mitochondrial complex I and II respiration. Furthermore, alterations in striatal gene and protein expression suggest a role for the microbiome in regulating mitochondrial protein metabolism and affecting oxidative stress. Analysis of the brain metabolome revealed the microbiome's imprint on region-specific changes in metabolite levels that may be linked to susceptibility to neuronal dysfunction. Motor testing of mice with or without microbiomes uncovered associations with striatal oxidative stress and enhanced progression of symptoms. Collectively, our data demonstrates that the gut microbiome influences mitochondrial functions in the brain of α-syn overexpressing mice, uncovering novel insights into environmental contributions to the pathophysiology of PD.

Disclosures: L.H. Morais: None. **L. Stiles:** None. **M. Ladinski:** None. **J. Boktor:** None. **S. Mahmoudiandehkordi:** None. **J. Jones:** None. **R. Tse:** None. **T. Chou:** None. **J. Trinh:** None. **R. Kaddurah-Daouk:** None. **S.K. Mazmanian:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.08

Topic: C.03. Parkinson's Disease

Support: NIH 123405

Title: Dj-1 regulates the neurite outgrowth in the dopaminergic neurons

Authors: *R. CHEN[1], C. MARTINEZ-ACEVES[1], E. GOUCHER[1], J. TANG[1], M. GRAHAM[1], W. CARTER[1], W. MANDEMAKERS[2], V. BONIFATI[2], E. JONAS[1];
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Abstract: Familial Parkinson's disease (PD) protein DJ-1 is required for stimulation dependent new protein synthesis in dopaminergic neuron (DN) processes. DJ-1 knockout DN culture and DJ-1 patient fibroblasts provide useful assays for early pathological alterations in PD patients' brains. We studied mitochondrial functional properties (morphology/ bioenergetics) in fibroblasts obtained from different DJ1 PD patients and aged-match healthy individual. We further investigated observed abnormalities in DNs isolated from the DJ-1 KO mouse midbrain.Mutations in DJ-1 and DJ-1 KO increase mitochondrial uncoupling and hamper extension and branching of dopaminergic neuronal processes, in part secondary to loss of the F1 complex of the ATP synthase, exposing the free c-subunit leak channel and causing ATP synthase inefficiency and low ATP levels. We found age dependent loss of ATP synthase β subunit was accelerated in the DJ-1 KO mice and isolated neurons, correlated with the loss of growth of the dopaminergic processes in isolated neurons and in vivo in the aged striatum, where we also observed loss of vesicle docking. In DJ-1 PD patients' fibroblasts, we further observed overall protein synthesis rates are low and synthesis rates of ATP synthase β subunit protein are dramatically decreased in patient cells and in DJ-1 KO brain tissue (baseline and after neuronal stimulation). We found that changes in ATP synthase stoichiometry are also associated with mitochondrial morphological changes including increased electron density in the mutant fibroblasts, a low number of cristae, a reduction in mitochondrial length and an increased number of total mitochondria per measured area.New protein synthesis is crucial for cells to respond to extra- and intracellular cues and to repair or remodel use-dependent damage at neuron growth cones and branch points. Protein synthesis rates were rescued in patient cells by ATP synthase β subunit overexpression, presumably by normalization of mitochondrial inner membrane coupling. DJ-1 is bound to ATP synthase β subunit and to its mRNA. We therefore suspected that the mechanism of decreased growth and branching of DJ-1 deficient DNs is related to a lack of DJ-1 trafficking of mRNAs to distal neurites. Our preliminary data show that indeed mRNA levels for ATP synthase β subunit, although not significantly different in the somata, are strikingly low in DJ-1 KO neuronal processes. These observations suggest a connection between ATP synthase complex stoichiometry, mitochondrial plasticity, protein synthesis rates, dopamine release sites and PD. These findings comprise a promising new strategy to develop therapies for PD patients.

Disclosures: R. Chen: None. **C. Martinez-Aceves:** None. **E. Goucher:** None. **J. Tang:** None. **M. Graham:** None. **W. Carter:** None. **W. Mandemakers:** None. **V. Bonifati:** None. **E. Jonas:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.09

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS117469
Wisconsin Alzheimer's Disease Research Center P30-AG062715

Title: Differential gene expression in whole blood as predictive biomarkers in a rat model of early-stage Parkinson disease

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Abstract: The molecular pathology of Parkinson disease (PD) in the early stages is poorly understood; as such, there are no specific predictive or diagnostic tests. To address early-stage biological questions, this work uses a validated animal model of early-onset and progressive PD, a genetic rat with a loss of function in the *Pink1* gene. Male *Pink1*-/- rats demonstrate progressive hindlimb and forelimb motor deficits by mid-to-late adulthood (8 months of age) as well as brainstem and muscle pathology including various markers of inflammation. The greater purpose of this work is to identify dysregulated pathways within the blood of young *Pink1*-/- rats (2-4 months of age) that appear prior to the onset of limb motor signs in this PD model. Specifically, we hypothesized that loss of *Pink1* would alter expression (pathways) in the blood, resulting in characteristic gene signatures that overlap between young (2-4mo) and old (14-16mo) animals and that these may constitute early predictors of limb motor deficits. To accomplish this, limb motor testing (open field test) was performed, immediately followed by the collection of whole blood RNA and serum protein samples from male rats: *Pink1*-/- rats (young, *n*=16; old, *n*=16) and wildtype (WT) controls (young, *n*=16; old, *n*=16). To determine age-related genetic differential expression, the Illumina® Total RNA-Seq TruSeq platform was used to profile differential expression of genes. Statistically significant genes (WT vs. *Pink1*-/- at each timepoint) were identified and ENRICHR gene enrichment was used to identify the top up-regulated biological pathways. Within the young rats, there were few up-regulated genes (*n*=19) but included interleukins and RAS protein activators. Older rats had an extensive list of up-regulated genes (*n*=553) and significant pathways including cytokine-cytokine receptor interaction, TNF signaling pathway, MAPK signaling pathway as well as necroptosis. Next, serum samples were probed for inflammation-related protein markers, CRP and TNF-α, and compared to open field limb motor behavior. The data showed that *Pink1*-/- rats have increased circulating levels of CRP and TNF-α compared to WT controls, that also increased with age.

These findings parallel to what is observed in human subjects with PD diagnoses. Within *Pink1*-/- rats, protein levels were significantly negatively correlated to total movement in the open field test; lesser motor patterns were associated with increased TNF-α levels. In general, these data identified several blood biomarkers and pathways that may predict PD limb movement in a rat model and are key candidates for comparative work to human datasets as well as for drug-repurposing.

Disclosures: S.A. Lechner: None. **J.M. Welsch:** None. **S.C. Gammie:** None. **C.A. Kelm-Nelson:** None.

Poster

P272. Parkinson's Genetics and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P272.10

Topic: C.03. Parkinson’s Disease

Support: R01ES027245
R01ES026892
R01NS100090
R01NS088206
Eugene and Linda Lloyd Endowment
Armbrust Endowment

Title: Mitochondrial Dysfunction and Proteasome Impairment Alters H3K27ac-enriched Enhancer and Transcriptional Signature by Inducing HAT/HDAC Imbalance in Parkinson’s Disease Models

Authors: *M. HUANG[1], D. LOU[2], Z. WANG[2], H. JIN[1], V. ANANTHARAM[1], A. KANTHASAMY[1], A. KANTHASAMY[1];
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Abstract: Abstract: Our recent epigenomic analysis using cell and animal models as well as human substantia nigra tissues revealed that epigenetic dysregulation plays a role in the etiopathogenesis of PD. However, a comprehensive understanding of how epigenetic histone marks modulate neurodegeneration remains elusive. Herein, through RNA-Seq and ChIP-Seq analyses on mitochondria-impaired cell models of PD, we confirmed that environmental chemicals that inhibit mitochondrial function induce histone H3K27ac deposition and alter neuronal enhancer activities and transcriptomic profile in dopaminergic neuronal cell models. Further mechanistic studies revealed a significant imbalance in HAT/HDAC, particularly increased expression of p300, and thereby contributing to hyperacetylation of H3K27. Mitochondrial inhibiting pesticide also inhibited proteasome activity, indicating the altered

bioenergy deficits can impair proteasome machinery in dopaminergic neuronal cells. Interestingly, proteasome inhibitor treatment shares a similar H3K27ac dysregulation of mitochondrial inhibition. Notably, the p300/H3K27ac inhibitor GNE-049 reversed mitochondrial impairment induced H3K27ac epigenetic dysregulation. Collectively, our results provide a comprehensive perspective on H3K27ac dysregulation following mitochondrial dysfunction and proteasome impairment and suggest that epigenetic-based personalized pharmacogenetic strategies can be developed for PD treatment. **Support:** R01ES027245, R01ES026892, R01NS100090 and R01NS088206, Eugene and Linda Lloyd Endowment and Armbrust Endowment.

Disclosures: M. Huang: None. **D. Lou:** None. **Z. Wang:** None. **H. Jin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None. **A. Kanthasamy:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.01

Topic: C.06. Neuromuscular Diseases

Title: Increased apoptotic cell death in a pediatric form of neurodegeneration associates with mitochondrial abnormalities

Authors: *C. MARIOLI[1,2], C. FIORUCCI[1], F. COLASUONNO[2,1], V. MAGLIOCCA[1,2], M. MUZZI[1], E. BERTINI[2], M. TARTAGLIA[2], C. COMPAGNUCCI[2], S. MORENO[1];
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Abstract: Riboflavin Transporter Deficiency (RTD) is a rare neurodegenerative disease affecting motor neurons (MNs), caused by loss-of-function mutations in *SLC52A3* and *SLC52A2* genes, encoding riboflavin transporters hRFT2 and hRFT3, respectively. Clinical presentation includes respiratory failure, progressive ponto-bulbar paralysis, bilateral sensorineural deafness, and muscle weakness[1]. As riboflavin (RF) is the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), it's abnormally low levels result in defective functionality of flavoproteins, many of which are involved in cellular bioenergetics, DNA repair, protein folding and cell survival processes[2].Previous work by our group demonstrated altered energy metabolism pathways depending on mitochondria and peroxisomes, accompanied by cytoskeletal derangement[3-5]. These studies took advantage of induced Pluripotent Stem Cells (iPSCs) technology, to produce *in vitro* models recapitulating RTD pathogenesis. In the present work, we studied MNs differentiated from patient-specific iPSCs to perform combined ultrastructural and confocal analyses, aimed at characterizing the cellular pathomechanisms associated to RTD. Our data obtained by TUNEL assay and immunofluorescence using specific apoptotic markers demonstrate increased RTD cell death

during neurogenesis. Consistently, Focused Ion Beam/Electron Microscopy (FIB/SEM) and conventional SEM showed profound alterations in patients' cells including: (i) abnormally short neurites with few branches, suggesting inability to properly differentiate into MNs; (ii) great abundancy of extracellular vesicles budding from the membrane, possibly indicating abnormal cell-cell communication and/or apoptotic blebs; (iii) conspicuous swellings along neurites, interpreted as sign of neurodegeneration; (iv) abnormal mitochondria characterized by few, deranged *cristae*, confirming persistence of mitochondrial damage after differentiation.Overall, our work contributes to the knowledge on the multiple cellular alterations associated to RTD neuronal phenotype, supporting a central role played by mitochondrial apoptosis in its pathogenesis, thus indicating potential targets for future therapies.[1]Green, P et al. Am. J. Hum. Genet. 2010; 86: 485.[2]Balasubramaniam, S et al. J. Inherit. Metab. Dis 2019; 42(4): 608.[3]Colasuonno, F et al. Oxid. Med. Cell. Longev 2020; 6821247 [4]Colasuonno, F et al. Antioxidants, 2020a; 9(12): 1252. [5]Niceforo, A et al. Dis Model Mech 2021; 14(2): dmm046391.

Disclosures: C. Marioli: None. **C. Fiorucci:** None. **F. Colasuonno:** None. **V. Magliocca:** None. **M. Muzzi:** None. **E. Bertini:** None. **M. Tartaglia:** None. **C. Compagnucci:** None. **S. Moreno:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.02

Topic: C.06. Neuromuscular Diseases

Title: Study of neuronal activity in co-culture of iPSC-derived astrocytes and motoneurons obtained by patients affected by riboflavin transporter deficiency.

Authors: *V. MAGLIOCCA[1,2], C. MARIOLI[1,2], E. BERTINI[2], M. TARTAGLIA[2], C. COMPAGNUCCI[2], T. PERSICHINI[1];
[1]Univ. of Roma Tre, Rome, Italy; [2]IRCCS Children's Res. Hosp. Bambino Gesù, Rome, Italy

Abstract: Riboflavin Transporter Deficiency (RTD) is a rare autosomal recessive disease that arises in childhood and is caused by mutations in the genes *SLC52A3* or *SLC52A2*, encoding for the human Riboflavin Transporters hRFT2 and hRFT3, respectively. The specific role of these transporters is to absorb the vitamin riboflavin (RF) [1]. RF is the precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), two biological cofactors which are involved in different metabolic oxidation-reduction reactions, especially in mitochondrial processes. For these reasons, RTD patients exhibit oxidative stress that impairs neuronal differentiation. Fortunately, RF administration in combination with other antioxidant treatments ameliorates the symptoms of the pathology in different patients [2]. To better understand the mechanisms involved in RTD pathogenesis, we used patient-derived induced pluripotent stem

cells (iPSCs). We have reprogrammed patients' fibroblasts into iPSCs and induced them to differentiate into motoneurons (MNs) and astrocytes (ASTROs). It is known that RTD MNs are shorter than healthy ones, have less branches and have impaired neuronal activity [3]. Since it has been highlighted the role of the antioxidant response of astrocytes in the protection against neurotoxicity, as well as in the improvement of morphology and neuronal activity, we aimed our study to understand if RTD MNs phenotype could ameliorate when RTD MNs are grown in co-culture with ASTROs. In this study, we obtained control (Ctrl) and patient ASTROs, which are positive for GFAP and EAAT2 and used them to perform RTD ASTROs-RTD MNs and Ctrl ASTROs-RTD MNs co-cultures, with and without direct contact. Neuronal function was evaluated in co-cultures by determining intracellular calcium (Ca^{+2}) levels following Ionomycin stimulation. The data obtained show that when in co-cultures, RTD MNs present intracellular Ca^{+2} levels similar to those of Ctrl MNs. We performed the co-culture experiments also by purifying ASTROs exosomes and adding them to the MNs cultures, to understand if the direct contact is required to ameliorate the RTD MNs phenotype or factors released by ASTROs can also influence the RTD phenotype. Our results support the hypothesis that ASTROs can improve neuronal activity and plasticity in RTD MNs compared to RTD MNs alone both by direct contact and by secreting important factors supporting neuronal health. We conclude that the ASTROs have a relevant role in the pathophysiology of RTD and we need more studies for a better understanding. 1. Callaghan B. J Inherit Metab Dis. 2019 Jul. 2.Marioli C. Int J Mol Sci. 2020 Oct 7. 3. Niceforo A. Dis Model Mech. 2021 Jan 19.

Disclosures: V. Magliocca: None. **C. Marioli:** None. **E. Bertini:** None. **M. Tartaglia:** None. **C. Compagnucci:** None. **T. Persichini:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.03

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01 GM127557

Title: Investigating the collapse of proteostasis in p97-related ALS using IPSC-derived motor neurons

Authors: *J. KLICKSTEIN, R. KHANNA, R. GANJI, M. RAMAN;
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Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by upper and lower motor neuron degeneration. In the majority of ALS cases, degeneration is preceded by hallmark molecular events including TAR DNA-binding protein 43

(TDP-43) mislocalization and endoplasmic reticulum (ER) stress.
In the last decade, p97/VCP/CDC48 (p97) has been discovered to cause ALS as well as other protein aggregation diseases. p97 is an ATPase that binds and extracts ubiquitinated substrates from membranes or multi-protein complexes. Through this action, p97 participates in a myriad of cellular processes including ER-associated degradation, autophagy, and mitophagy, among others. p97 is targeted to its functions by protein adaptor interaction with the N-terminal domain. Studies have shown that p97 mutations alter adaptor interaction suggesting that mistargeting of p97 may lead to disease; however, these studies have not been performed in motor neurons. As a hub for cellular processes, it remains unknown which functions of mutant p97 are impaired. Uncovering the specific pathways altered in p97-related ALS will provide insight into salient processes that engender ALS pathogenesis.
Methods: To study the role of p97 in ALS, we have developed a novel isogenic model p97-related ALS using CRISPR gene editing to introduce the most common mutations: R155H and R159H. All studies have been performed with two clones to control for potential off-target effects.
Results: Using a novel differentiation paradigm, we can produce spontaneously active, mature motor neurons within 3 weeks. This protocol produces TUJ1 and ChAT positive cells by day 20 that exhibit spontaneous action potentials. p97 mutations do not alter the efficiency of differentiation; however, these mutations reduce the proportion of spontaneously active neurons. Additionally, mutant neurons display induction of ATF4, increased LC3b, and TDP-43 mislocalization. Using immunoprecipitation, we found altered adaptor association to p97 which is dependent on cell type (IPSC vs motor neuron) with some adaptors increasing association and others decreasing. We have performed quantitative proteomics on motor neurons and IPSCs (9-plex with 3 replicates per genotype each cell type). Network analysis revealed significantly altered cellular processes and functions that were unique to each cell type.
Conclusions: Our novel isogenic model of mutant p97 ALS recapitulates the phenotypes seen in ALS patients. Altered adaptor association and cellular processes due to p97 mutations are cell-type specific highlighting the importance of using a physiological model.

Disclosures: J. Klickstein: None. **R. Khanna:** None. **R. Ganji:** None. **M. Raman:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.04

Topic: C.06. Neuromuscular Diseases

Title: An allelic series of SCYL1 mutations identifies molecular interactions essential for function

Authors: *A. CASSIDY[1], E. KULIYEV[2], D. B. THOMAS[1], S. GINGRAS[3], H. CHEN[1], S. PELLETIER[1];
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Abstract: SCYL1 is a multidomain protein thought to regulate several essential cellular functions including membrane protein trafficking, nucleocytoplasmic shuttling of tRNAs and transcription via interactions with several molecular complexes. In both humans and mice, inactivating *SCYL1* mutations cause a multisystem disorder characterized by recurrent episodes of liver failure, growth retardation and progressive motor dysfunction resulting from the loss of spinal motor neurons. However, the molecular mechanisms underlying SCYL1 deficiency syndrome has remained elusive. Here we report the characterization of an allelic series of genetically engineered mice harboring *Scyl1* separation-of-function mutations disrupting individual or several molecular interactions. Our results demonstrate an essential role for the proper localization of SCYL1 to the ER-Golgi Intermediate Compartment and its homo-oligomerization for function in vivo and suggest that defective mRNA trafficking and/or metabolism represents a major disease mechanism in SCYL1 deficiency syndrome. Mice expressing mutant forms of SCYL1 that disrupt SCYL1-tNRA interaction, oligomerization, or subcellular localization exhibited growth retardation and developed motor dysfunction. On the other hand, although defective protein trafficking and faulty transcription were viewed as major disease mechanisms in SCYL1 deficiency syndrome, our findings do not support this notion. Mice harboring mutations in *Scyl1* that prevented interaction between SCYL1 and the membrane coat protein complexes COPI, COPII or several other factors involved in protein trafficking, and mice expressing a form of SCYL1 lacking transcriptional activity showed no overt abnormalities. Together, our findings identify key molecular interactions essential for SCYL1 function in vivo and provide the foundation for further studies exploring the potential role of SCYL1 in regulating tRNA trafficking and/or metabolism.

Disclosures: A. Cassidy: None. **E. Kuliyev:** None. **D.B. Thomas:** None. **S. Gingras:** None. **H. Chen:** None. **S. Pelletier:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.05

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS115977
NIH Grant R01NS118177

NIH Grant R01NS28785
NIH Grant R21AG068597
Lisa Dean Moseley Foundation
Spastic Paraplegia Foundation
CURE Grant SAP Number: 4100062203

Title: Spast-based hereditary spastic paraplegia is caused by gain-of-function cytotoxicity but exacerbated by haploinsufficiency.

Authors: *E. PIERMARINI[1], S. AKARSU[2], T. CONNORS[1], C.-H. CHENG[3], B.-B. KOO[3], N. HUA[3], M. KNEUSSEL[4], M. A. LANE[1], G. A. MORFINI[5], A. KARABAY[2], P. W. BAAS[1], L. QIANG[1];
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Abstract: The plurality of cases of Hereditary Spastic Paraplegia (HSP), a disease in which dieback degeneration of the corticospinal tracts leads to gait deficiencies, results from mutations of *SPAST,* the gene that encodes spastin, a microtubule-severing protein. The hSPAST-C448Y mouse, which expresses both wild-type mouse and mutant human spastins, displays these disease hallmarks. However, the corticospinal tracts do not show abnormal axonal swellings that are typical in postmortem tissues of patients. Here, the hSPAST-C448Y mouse was crossbred with a Spast-knockout mouse that displays axonal swellings but no gait deficiency or corticospinal dieback. The crossbred animals displayed axonal swellings, and also earlier onset and more extreme gait deficiencies and corticospinal dieback than the hSPAST-C448Y mouse. The spinal cords of the crossbred mice showed changes in microtubule modifications and HDAC6 activity similar to the hSPAST-C448Y mice, not the knockout mouse. Collectively these results indicate that gain-of-function of toxicity is the etiology of *SPAST*-based HSP (also called HSP-*SPG4)*, but that haploinsufficiency contributes to the disease by making the corticospinal tracts more vulnerable to the toxicity of the mutant protein. An advanced type of Diffusion Magnetic Resonance Imaging identified the dieback degeneration in isolated spinal cords, portending a biomarker for assessing the status of patients.

Disclosures: E. Piermarini: None. **S. Akarsu:** None. **T. Connors:** None. **C. Cheng:** None. **B. Koo:** None. **N. Hua:** None. **M. Kneussel:** None. **M.A. Lane:** None. **G.A. Morfini:** None. **A. Karabay:** None. **P.W. Baas:** None. **L. Qiang:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.06

Topic: C.06. Neuromuscular Diseases

Support: DARPA Grant D20AC00002

Title: Chronic recording of spontaneous and evoked muscle activity from the VML wound model of Canine Biceps Femoris during recovery from injury

Authors: *M. JAVIDI[1], S. A. JOHNSON[2,3], R. CRUM[3], S. F. BADYLAK[2,3,4], D. J. WEBER[1]; [1]Dept. of Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [2]McGowan Inst. for Regenerative Med., [3]Sch. of Med., [4]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Abstract Volumetric muscle loss (VML) is common for soldiers wounded in overseas conflicts. Recovery of muscle function from injury requires regeneration of muscle and nerve tissues and reinnervation of the new muscle tissue. Chronic nerve stimulation at sub levels of motor threshold has been suggested as an effective approach to accelerate muscle and nerve regeneration after injury. In the current study, a volumetric muscle model (VML) (approximately 70% of muscle was removed) was generated in Biceps Femoris of a Canine and chronic nerve stimulation started on post-op day 1 to accelerate muscle regeneration and innervation. An implanted pulse generator was programmed to deliver nerve stimulation twice daily for 10 days post-op by stimulating of the sciatic nerve at intensity of 80% motor threshold (~400 μA catholdal pulses at 200 μs pulse-width). Stimulation pulses were delivered at 10 Hz and applied for 1(h) per day in two 30 (min) sessions, 12 hours apart. To evaluate muscle function over time during recovery from injury, EMG was recorded at 4 different locations in and around the wound bed. One electrode was placed in an uninjured portion of the muscle and three other electrodes were in proximal, distal, and lateral edges of the wound bed. Compound muscle action potentials (CMAPs) were evoked by electrical stimulation at the sciatic nerve. Recruitment curves for each of the 4 EMG electrodes were measured on post-op days 3, 7, 10, and 14. EMG also was collected during normal walking of the animal, which was done twice per day for 5 (min) and at multiple timepoints throughout 24-hour a day. Results of recruitment curves indicated gradual improvement in muscle activation in the bed and edges of wound, with the strongest activation occurring at post-op day 14, which was the last day tested. The data collected during the walking experiments shows increases in both activity level and EMG power over the 14-day recovery period. Similar improvements in EMG and activity level were observed in the daily samples taken at random intervals throughout the day. Specifically, on post-op days 2, 5 and 8 animal indicated significant signs of recovery when the average of normalized mean RMS of four leads increased by 66, 96 and 119 percent for walking tests and 118, 112 and 227 percent for random EMG measurement tests in compared to first post-op day. These preliminary results demonstrate long term nerve stimulation and recording using fully implanted devices that permit chronic control and sensing of neuromuscular activity. This animal model and implant system will be used to develop a closed-loop bioelectronic interface for monitoring and enhancing wound healing after volumetric muscle loss.

Disclosures: M. javidi: None. **S.A. Johnson:** None. **R. Crum:** None. **S.F. Badylak:** None. **D.J. Weber:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.07

Topic: C.06. Neuromuscular Diseases

Support: Arizona Alheimers Consortium
Arizona Department of Health ADHS18-198843

Title: Cortical and spinal cord ALS-and FTD-like neurodegenerative effects following a traumatic brain injury in a non-genetic animal model

Authors: *G. BJORKLUND[1], J. WONG[1], R. BOWSER[2], S. STABENFELDT[1];
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Abstract: Traumatic brain injuries (TBIs) are a significant health problem worldwide with over 27 million cases reported annually. Primary injuries from a TBI cause immediate tissue and cellular damage followed by secondary injury sequalae that can provide opportunities for therapeutic intervention to improve outcomes in the minutes and years following an injury. These secondary injury processes, described as delayed and continual tissue damage caused by consecutive pathological processes, may contribute to chronic conditions that result in permanent functional and cognitive disabilities and a highly increased risk for the development of neurodegenerative disorders (NDDs). TBIs and repetitive brain injuries are increasingly associated with NDDs such as Alzheimer's disease (AD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). In addition to TBIs, a variety of additional factors such as intense physical activity, chemical exposure, heavy metals exposure, and smoking are some examples of recognized causes for an increased risk of developing NDDs. In this study, we examined the neurodegenerative effects of a TBI on neurons in the forebrain and cervical spinal cord distal to the site of initial injury. Adult male C57BL/6J mice with no genetic predisposition to neurodegenerative disease were subjected to a unilateral controlled cortical impact on the primary motor cortex. Post-injury analysis includes short-term timepoints at 7, 14, and 28 days post injury (DPI) and long-term timepoints at 120 and 180 DPI. Tissue samples from the forebrain and cervical spinal cord were analyzed for pathologic biomarkers of FTD and ALS via immunohistochemistry. Specifically, neurons were examined for TAR DNA Binding Protein 43 (TDP-43) translocation from the nucleus and cytosolic accumulation with the presence of ubiquitination and hyperphosphorylation. These cellular pathologies of TDP-43 are strongly associated with AD, ALS, and FTD. Current results at the short-term timepoint indicate a significant mislocalization of nuclear TDP-43 from NEUN positive neurons in the forebrain and cervical spinal cord accompanied by an accumulation of cytosolic and ubiquitinated TDP-43. Analysis at the long-term time points indicate no difference between control mice and TBI mice in TDP-43 nuclear mislocalization. However, preliminary results suggest a decrease in total NEUN positive cells of the spinal cord in the TBI mice. As TDP-43 nuclear mislocation and

cytosolic aggregation is a hallmark of ALS and present in the majority of FTD cases, these results support a possible link between TBI and the development of FTD- and ALS-like neurodegenerative diseases.

Disclosures: G. Bjorklund: None. **J. Wong:** None. **R. Bowser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder Iron Horse Diagnostics. **S. Stabenfeldt:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.08

Topic: C.06. Neuromuscular Diseases

Title: Loss of WWOX and mutations in the gene cause mitochondrial dysfunction in amyotrophic lateral sclerosis

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Abstract: To date, there are no effective treatments for amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting motor neurons that leads to death within 2-5 years from diagnosis. This is due to our limited knowledge of the disease-causing mechanisms in ALS, highlighting the importance of understanding and identifying the molecular events underlying motor neuron degeneration. One candidate that may be involved in ALS pathogenesis is the WW domain-containing oxidoreductase (WWOX), a protein that is involved in neurodegenerative processes. Specifically, WWOX is decreased in post-mortem brains from Alzheimer's disease patients and mutations in the *WWOX* gene cause seizures, epilepsy and cerebellar ataxia. WWOX is involved in regulating the activity of the mitochondrial electron transport chain (mtETC) and in reducing oxidative stress through its short-chain dehydrogenase/reductase (SDR) domain. Interestingly, mitochondrial dysfunction and oxidative stress are key and early pathogenetic event in ALS. Therefore, we hypothesized that alterations in WWOX may contribute to mitochondrial dysfunction in ALS thus worsening disease progression. Our findings reveal a significant decrease in WWOX levels in ALS post-mortem motor cortex (mCTX) that correlates with faster disease progression, suggesting that loss of WWOX may play a pathogenic role in ALS. Furthermore, we have demonstrated that knocking down WWOX using siRNA decreases reactive oxygen species (ROS) in human neuroblastoma SH-SY5Y cells further supporting a link between loss of WWOX and increases in oxidative stress in ALS. We also identified several deleterious variants in *WWOX* gene in ALS cases by analyzing Project

MinE and ALSKP data sets. One of these ALS specific mutations, a stop codon mutation located at amino acid 261 in the mitochondrial binding region of WWOX SDR domain, decreases ATP levels, increases ROS levels and causes cell death in SH-SY5Y cells. These results were consistent with our findings revealing a decrease in two mtETC proteins, the mitochondrial membrane ATP synthase of complex V (ATP5A) and the cytochrome c oxidase of complex IV (COX II) in ALS mCTX. Together, our results suggest that loss of WWOX as well as mutations in the gene may cause and exacerbate mitochondrial dysfunction in ALS.

Disclosures: G. Sadri-Vakili: None. **T. Petrozziello:** None. **S. Kim:** None. **S. Farhan:** None. **J. Roy:** None. **K. Vakili:** None. **J. Berry:** None. **J. Walker:** None. **R. Mouro-Pinto:** None.

Poster

P294. Motor Neuron Diseases

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P294.09

Topic: C.06. Neuromuscular Diseases

Support: A Long Swim

Title: Comprehensive analysis of molecular pathways involved in health of corticospinal motor neurons deficient of Alsin function

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Abstract: Corticospinal motor neurons (CSMN) reside in cerebral cortex and they play an important role in initiation and modulation of voluntary movement by collecting and integrating signals from different regions of the cerebral cortex and relay that information to their spinal cord targets. Degeneration of CSMN manifests in the development of hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS) and amyotrophic lateral sclerosis (ALS). Several gene mutations are implicated with the onset of ALS, leading to motor neuron vulnerability, progressive degeneration, and dysfunction of motor neuron circuitry. Alsin is a member of a small GTPase gene family that are involved in neurite outgrowth, endosomal trafficking, and cytoskeleton maintenance. Mutations in the Alsin gene have been shown to manifest into juvenile ALS onset. The present study investigates molecular pathways implicated in the health of CSMN that are devoid of Alsin function. We previously generated and characterized UCHL1-eGFP mice in which CSMN are genetically labelled with stable eGFP expression allowing their visualization andprecise investigation. The UCHL1-eGFP mice were crossbred with AlsinKO mice to generate AlsinKO-UeGFP mice, allowing purification of AlsinKO CSMN via FACS-mediated approaches. We performed transcriptomics (RNA-Seq) and

proteomics analysis on pure populations of CSMN isolated from AlsinKO-UeGFP and AlsinWT-UeGFP mice. Our ongoing study reveals key molecular and cellular pathways that are differentially regulated due to the absence of Alsin function. The genes and proteins involved in fatty acid metabolism, immune response, and mitochondrial function were found to be differentially regulated. Our study reveals the molecular and cellular mechanisms that are involved in neuronal dysfunction in the absence of Alsin.

Disclosures: I. Aguilar-Wickings: None. **M. Gautam:** None. **Y.A. Goo:** None. **P. Ozdinler:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.01

Topic: B.11. Glial Mechanisms

Support: IBRO Travel Grants
PAPIIT (IN213818)
DGAPA-UNAM

Title: The amyloid-β (25-35) induces changes in the expression of galectin-9 and TIM3 implicated in the modulation of the neuroinflammation in rats

Authors: *E. RAMÍREZ HERNÁNDEZ[1], O. A. GUERRERO MARÍN[2], L. VALENCIA GIL[2], L. HERNÁNDEZ ZIMBRÓN[2], M. NAVA CATORCE[2], E. ZENTENO[2];
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Abstract: Background: Neuroinflammation has been proposed as a common factor and one of the main inducers of neuronal degeneration in Alzheimer's disease (AD) [1,4]. Galectins are a group of β-galactoside-binding lectins, that play an important role in the immune response, adhesion, proliferation, differentiation, migration, and cell growth. Up to 15 members of the galectin's family have been identified; the expression of galectin-9 and receptor TIM3 is considered a key factor in modulation of the inflammatory response [2]. The administration of $A\beta_{25-35}$ peptide into the *lateral ventricles* of rats can cause memory impairment by the activation of the inflammatory response [3]. Despite all these studies, the specific molecular mechanisms involved in this inflammatory process remains unclear. Galectin-9 (Gal-9) and TIM3 are associated with the modulation of astrocyte and microglia activation under various inflammatory conditions. The aim of this study was to evaluate if the administration amyloid-β (25-35) into *lateral ventricles* of rats increase the expression of galectin-9 and TIM3 implicated in the modulation of the neuroinflammation. **Methods:** The vehicle or $A\beta_{25-35}$ peptide (0.1 µg/µL) was

bilaterally administered in the *lateral ventricles* of the rat, and control group. On day 30, the hippocampus and dentate gyrus were assessed for immunohistochemical and western blot assays for immunodetection of astrocytes (GFAP), microglia (Iba1), Gal-9 and TIM3. **Results:** Our results show that the administration of the $A\beta_{25-35}$ peptide increases the reactivity glial cells in the hippocampus of $A\beta_{25-35}$ treated rats in comparison to the control group. This result is associated with an increase in the expression of Gal-9 and TIM3 that was detected predominantly in the cells of microglia respect to the control group. **Conclusion:** Gal-9 and TIM3 is mainly produced by activated microglia that can attenuate injuries in the peak phase of the inflammatory response, in neurodegenerative process induced by $A\beta_{25-35}$ peptide. Therefore, we suggest that Gal-9 and TIM3 are involved in the inflammatory process induced by the administration of the $A\beta_{25-35}$.1 Heneka MT et al., 2014. Nat. Rev. Immunol. 14: 463-477.2 Rabinovich GA et al., 2002. Trends Immunol. 23: 313-320.3 Ramírez E et al., 2019. Neuropeptides. 74:11-23.4 Zussy C et al., 2011. Am. J. Pathol. 179: 315-334.

Disclosures: E. Ramírez Hernández: None. **O.A. Guerrero Marín:** None. **L. Valencia Gil:** None. **L. Hernández Zimbrón:** None. **M. Nava Catorce:** None. **E. Zenteno:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.02

Topic: B.11. Glial Mechanisms

Support: RF1 AG051495
RF1 AG050597 203

Title: Trem2 splicing generates different species of soluble trem2 that inhibit ltp through $gaba_a$ receptors

Authors: *M. MOUTINHO, I. CORONEL, G. V. DI PRISCO, A. P.-Y. TSAI, S. S. PUNTAMBEKAR, K. NHO, B. K. ATWOOD, G. E. LANDRETH;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: TREM2 is a transmembrane receptor expressed by myeloid cells which acts to regulate their immune response. Genetic studies have demonstrated that TREM2 variants confer dramatically elevated risk for Alzheimer's disease (AD). In the AD brain, TREM2 expression is induced in a subset of plaque-associated microglia and regulates their response to amyloid and tau pathology. The *TREM2* gene yields three transcripts, $TREM2^{230}$ encoding the full-length transmembrane receptor, and the alternatively spliced isoforms $TREM2^{222}$ and $TREM2^{219}$ with distinct C-termini. Soluble TREM2 (sTREM2) levels in CSF fluctuate in function of the AD clinical stage and may serve as an novel biomarker for disease progression. sTREM2 is

generated by proteolytic cleavage of TREM2^{230} receptor, releasing its ectodomain (sTREM2) to the extracellular milieu. *TREM2*^{222} and *TREM2*^{219} represent a significant fraction of *TREM2* transcripts in the human brain, and since they lack a transmembrane domain, they are postulated to be secreted and function as an additional source of sTREM2. However, *TREM2*^{222} and *TREM2*^{219} translation into protein and secretion from cells has not been assessed. Although few studies have explored the biological function of sTREM2 at supra-physiological levels, the source of sTREM2 species and their endogenous role are still unclear. Our analysis of the AMP-AD dataset revealed that *TREM2*^{230}, *TREM2*^{222} and *TREM2*^{219} transcripts are increased in the brains of AD patients. We observed similar increases by qPCR in the brain of the AD mouse model 5xFAD expressing human *TREM2*. Analysis of HEK cells transfected with plasmids encoding *TREM2* isoforms revealed that *TREM2*^{222} and *TREM2*^{219} are secreted into the extracellular milieu, in contrast to the shedding of TREM2^{230}. This is the first experimental evidence that splice isoforms of human TREM2 are translated and directly secreted from cells. These observations suggest that increased expression of *TREM2*^{222} and *TREM2*^{219} in AD may lead to higher production of sTREM2. Interestingly, sTREM2 has been shown to bind to neurons, which suggests they may exert direct effects on neuronal function. We purified sTREM2 derived from TREM2^{230}, TREM2^{222} and TREM2^{219}, and incubated murine brain slices followed by LTP analysis. All three forms of sTREM2 abolished LTP induction, and this effect was inhibited by the $GABA_A$ receptor antagonist picrotoxin. These data support the existence of a heterogeneous pool of sTREM2 species in the brain, which act to inhibit LTP through $GABA_A$ receptors. Given that sTREM2 levels are linked to AD pathogenesis and progression, our findings that sTREM2 species interfere with LTP might help to decipher their role in disease.

Disclosures: M. Moutinho: None. **I. Coronel:** None. **G.V. Di Prisco:** None. **A.P. Tsai:** None. **S.S. Puntambekar:** None. **K. Nho:** None. **B.K. Atwood:** None. **G.E. Landreth:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.03

Topic: B.11. Glial Mechanisms

Support: NIH Grant R56AG067489
NIH Grant R01AG065541
NIH Grant R01AG071465
NIGMS Grant T32GM007752

Title: Altered neuronal ratios and microglial activation in aging Down syndrome brains revealed by snRNA-seq

Authors: *C. PALMER[1], C. LIU[1], W. ROMANOW[2], M.-H. LEE[3], J. CHUN[2];
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Abstract: Down Syndrome (DS) – trisomy of human chromosome 21 (HSA21) – is characterized by lifelong cognitive impairments and development of the neuropathological hallmarks of Alzheimer's disease (AD). The cellular and molecular modifications responsible for these effects are not understood. Here we conducted single-nucleus RNA-sequencing (snRNA-seq) on a total of 29 DS and non-DS control human prefrontal cortex samples. In DS, inhibitory neurons were overrepresented vs. excitatory neurons across all ages profiled. Microglia displayed profiles of aging and activation preceding and during AD neuropathology. Notably, increased expression of C1q complement genes associated with dendritic pruning was observed in young DS brains, as well as a general lack of homeostatic microglia at all ages in DS. A set of HSA21 transcription factors were implicated in driving this constitutive microglial activation. These results highlight global cellular and transcriptomic differences as novel features of the aging DS brain.

Disclosures: C. Palmer: None. **C. Liu:** None. **W. Romanow:** None. **M. Lee:** None. **J. Chun:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mosaic Pharmaceuticals.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.04

Topic: B.11. Glial Mechanisms

Support: R01NS110825
R01NS088627

Title: The Role of Voltage-gated Proton Channel Hv1 in Glioma Progression

Authors: *J. ZHENG[1,2], L. WANG[1,2], E. GODDERY[2,3], S. ZHAO[1], K. AYASOUFI[3], C. FAIN[3,2], Y. YING[1], T. CHEN[1], L. LI[1], A. JOHNSON[1,3,4], L.-J. WU[1,3,5];
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Abstract: In glioma, tumor-associated myeloid cells (TAMCs), including microglia, monocytes, macrophages, dendritic cells and granulocytes, are massively recruited and can comprise up to 50% of the total tumor mass. These diverse myeloid populations foster glioma progression by promoting angiogenesis, secreting soluble mediators to malignant cells, and suppressing local

immunity. Restraining TAMC recruitment by the glioma has beneficial effects on survival. Here, we identified that the voltage-gated proton channel, Hv1, regulates myeloid cell infiltration during glioma progression. Hv1 is a unique ion channel with high selectivity for protons. Hv1 is selectively expressed in microglia in the mouse central nervous system, and some peripheral immune populations, including monocytes, macrophages, neutrophils, and B cells. Using a GL261 mouse glioma model, we found that mice lacking Hv1 (*Hv1*$^{-/-}$) exhibit reduced tumor burden and prolonged survival compared to the wild-type mice. The prolonged survival in *Hv1*$^{-/-}$ mice is associated with slower glioma proliferation. RNA sequencing results suggest that Hv1 depletion modulate immune responses to glioma, including monocyte chemotaxis. We further performed flow cytometry to identify that *Hv1*$^{-/-}$ glioma mice have reduced monocyte and macrophage infiltration, and increased number of PD-1^{+}CD4^{+} T cells. As a result, we combined anti-PD-1 treatment with Hv1 knockout, and found that approximately 30% of *Hv1*$^{-/-}$ mice can be cured. Together, our results demonstrated that Hv1 controls tumor myeloid infiltration and shed light on the possibility of new treatment for glioma by blocking Hv1.

Disclosures: J. Zheng: None. **L. Wang:** None. **E. Goddery:** None. **S. Zhao:** None. **K. Ayasoufi:** None. **C. Fain:** None. **Y. Ying:** None. **T. Chen:** None. **L. Li:** None. **A. Johnson:** None. **L. Wu:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.05

Topic: B.11. Glial Mechanisms

Support: RO1 NS106597-01A1
RO1 EY026629

Title: Intravital microscopy reveals dynamic hypercoagulation contributes to microhemorrhage and microgliosis in experimental Cerebral Malaria

Authors: *O. D. SOLOMON[1], P. P. VILLARREAL[2], N. D. DOMINGO[3], L. F. OCHOA[4], L. PUEBLA CLARK[3], F. AUSSENAC[3], A. E. CARDONA[6], R. STEPHENS[3], G. VARGAS[5]; [1]Dept. of Human Pathophysiology/ Translational Med., [2]Dept. of Biomed. Engin. and Imaging Sci. Group, [3]Dept. of Intrnl. Medicine, Div. of Infectious Dis., [4]Dept. of Neurosci., [5]Dept. of Neuro, Cell Biol. & Anat. and Biomed. Engin. and Imaging Sci. Group, The Univ. of Texas Med. Br., Galveston, TX; [6]Dept. of Biol., The Univ. of Texas At San Antonio, San Antonio, TX

Abstract: Cerebral malaria (CM) is the most deadly pathology of *Plasmodium falciparum* infection, which primarily impacts children in sub-Saharan Africa. Survivors experience neurological sequelae including behavioral & cognitive defects. In *Plasmodium* infection in mice

deficient in IL-10, we showed that hyperinflammation drives deposition of fibrin(ogen) deposits that block the cerebral vasculature, as in human CM, and that glia are reactive. Anti-coagulants prevented mortality in mice and reduced gliosis. Using intravital multiphoton microscopy, we show a dynamic interplay of microglia directly with the congested vasculature. While parasite infection is limited to blood cells, this exchange suggests a mechanism of translation of inflammatory signals between the vasculature and parenchyma in experimental CM. To evaluate microglia interactions, with the congested vasculature, intravital microscopy was performed through a thin skull preparation on $IL10^{-/-}$ $CX3CR1^{GFP/-}$ $CCR2^{RFP/-}$ mice, highlighting resident microglia, and recruited CCR2+ leukocytes and their interactions with fluorescently labeled fibrinogen in eCM. These studies reveal that early in infection (3 days post infection (dpi)), coagula are transient with evidence of temporary flow disruption and emboli, while at 7 dpi thrombi show permanence and fully occlude flow. Microhemorrhages occur at 7dpi, occur proximally to thrombi and result in significant extravasation of luminal fibrinogen, which deposits in puncta in the parenchyma. We previously observed that reactive astrocytes localize to sites of clotting, and now observe a significant increase in vessel associated microglia (VAM) in infection compared to control (40% vs. 20%, $p<0.05$) at 7 dpi. VAM are primarily amoeboid, a reactive feature, compared to those away from vessels further into the parenchyma which have ramified, surveying morphology. Both flow cytometry and microscopy show that activated microglia contain fibrin(ogen) (IVM % average: 91% vs. 3% colocalization) with microscopy showing dynamic uptake by microglia processes. Microglial activation is further demonstrated by upregulation of antigen presentation molecules, MHCII and CD86. Finally, in vivo imaging reveals a qualitative increase in vasoconstriction & vasodilation. The overall picture that emerges is a dynamic progression of coagulation that ultimately leads to both microhemorrhage and the attraction of activated microglia to the vasculature, with evidence for dynamic uptake of fibrinogen by microglia, suggesting potential stimuli of activation, and supporting the interplay between hypercoagulation and neuroinflammation in eCM.

Disclosures: O.D. Solomon: None. **P.P. Villarreal:** None. **N.D. Domingo:** None. **L.F. Ochoa:** None. **L. Puebla Clark:** None. **F. Aussenac:** None. **A.E. Cardona:** None. **R. Stephens:** None. **G. Vargas:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.06

Topic: B.11. Glial Mechanisms

Support: NINDS, R01NS088627
NINDS, R01NS112144

Title: Activating microglial Gi signaling by chemogenetics increases anxiety-like behaviors.

Authors: *S. ZHAO[1], J. ZHENG[1,2], L. WANG[1,2], Y. YING[1], A. DHEER[1], L.-J. WU[1,3,4];
[1]Dept. of Neurol., Mayo Clin., rochester, MN; [2]Mayo Clin. Grad. Sch. of Biomed. Sci., [3]Dept. of Immunol., Mayo Clin., Rochester, MN; [4]Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: G protein-coupled receptors (GPCRs) regulate a wide array of physiological responses by modulating second messengers in all types of cells. Microglial GPCRs have been implied in regulating neuronal activity, neurogenesis, neuroplasticity, pain, and synaptic pruning etc. We previously identified that knockout of a microglial Gi-coupled GPCR, P2Y12, increased anxiety-like behaviors in mice. However, the consequence of activating microglial Gi protein-coupled GPCR in anxiety is still unknown. To this end, we took advantage of $CX3CR1^{creER/+}$:$R26^{LSL\text{-}hM4Di/+}$ mice to exclusively activate microglial Gi signaling by administrating CNO. Two-photon imaging showed microglia decreased ATP induced chemotaxis but did not alter morphology and surveillance in response to CNO injection. Furthermore, both male and female mice increased anxiety-like behaviors 30 min after activating microglial Gi signaling. C-fos mapping in 3 anxiety related brain regions indicates the basolateral amygdala (BLA) nuclei-specific contribution. These results suggest that activating microglial Gi-coupled GPCR increases neuronal excitability and anxiety-like behaviors in mice.

Disclosures: S. Zhao: None. **J. Zheng:** None. **L. Wang:** None. **Y. Ying:** None. **A. Dheer:** None. **L. Wu:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.07

Topic: B.11. Glial Mechanisms

Support: NIH Grant HL135635
VA Merit Award VX004294

Title: Maternal hypercaloric diet triggers astrogliosis and microglia reactivity in male mouse offspring

Authors: *C. BERDASCO[1], M. ELGAZZAZ[1], E. D. LAZARTIGUES[2];
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Abstract: It is well known that, in adults, obesity is associated with a state of inflammation throughout the body, including in the brain. Also, it is known that parental illnesses can impact the offspring. The objective of this work was to determine whether maternal consumption of a hypercaloric diet (HCD) during pregnancy and lactation could affect the inflammatory status of

the brain in the progeny. For this work, C57BL/6 mice females (4 months) were placed on either HCD or regular diet (RD) for 1 month prior to mating. Each litter was restricted to 6 individuals and the pups were separated after 21 days and fed RD or HCD for 9 weeks, thus creating 4 diet conditions: RD/RD, RD/HCD, HCD/RD and HCD/HCD. Male progeny brains (n=4/group) were used for immunohistochemistry against GFAP (glial fibrillary acidic protein) to identify reactive astrocytes (number and expression level) and Iba1 (ionized calcium-binding adapter molecule 1) to identify reactive microglia (expression level, activation score and morphology). HCD consumption in the perinatal phase or in adulthood, was associated with an increase in the number of $GFAP^+$ cells (HCD/RD: 12.4 ±0.5, RD/HCD: 10.1 ±0.5 and HCD/HCD: 11.9 ±0.6 compared to RD/RD: 7.6 ±0.3 cells/area, one-way ANOVA, $p<0.05$) and the overall GFAP expression level (HCD/RD: 3.25 ±0.19, RD/HCD: 2.31 ±0.11 and HCD/HCD: 2.81 ±0.08 compared to RD/RD: 1.79 ±0.06 A.U. in Integral Optical Density/Astrocyte, one-way ANOVA, $p<0.05$). Similarly, all HCD groups showed an increase in the expression level of Iba1 (HCD/RD: 7.31 ±0.5, RD/HCD: 6.45 ±0.4 and HCD/HCD: 8.29 ±0.6 compared to RD/RD: 0.37 ±0.3 A.U. in IOD/Microglia, one-way ANOVA, $p<0.05$). Also, all groups fed with HCD showed an increase in the microglia activation score (HCD/RD: 3.27 ±0.3, RD/HCD: 3.72 ±0.3 and HCD/HCD: 4.61 ±0.3 compared to RD/RD: 1.65 ±0.2 A.U. in microglia activation score, one-way ANOVA, $p<0.05$) in comparison to RD/RD group. Finally, all the mice fed with HCD showed a decrease in ramified microglia (HCD/RD: 20.2 ±3.5, RD/HCD: 15.5 ±3.4 and HCD/HCD: 15.8 ±3.6 compared to RD/RD: 53.9 ±5.5 in microglia morphology (%), two-way ANOVA, $p<0.05$). Specifically, HCD/RD group showed an increase in the hyper-ramified microglia (Hyper-ramified: 48.6 ±5.4 compared to Ramified: 20.2 ±3.5, Bushy: 12.8 ±3.4, and Ameboid: 18.3 ±3.7 in microglia morphology (%), two-way ANOVA, $p<0.05$). These data show that HCD-exposure during the perinatal period predisposes the offspring to neuroinflammation which as evidenced by the activation of microglia and astrocytes.

Disclosures: C. Berdasco: None. **M. Elgazzaz:** None. **E.D. Lazartigues:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.08

Topic: B.11. Glial Mechanisms

Support: Bluebird Bio partial funding
ANR-15-CE37-0004-01
ANR-15-NEUC-0004
Laboratory for Excellence "Revive" Post-Doctoral Fellowship

Title: Hematopoietic stem cell transplantation busulfan chemotherapy causes complete microglia senescence driving macrophage brain engraftment

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Abstract: Hematopoietic stem cell transplantation (HSCT) is a treatment for various malignant and non-malignant blood diseases with over 50,000 procedures annually worldwide. Yet, despite its prevalence, it is still unclear how HSCT affects the brain. Here we performed HSCT in mice using the myeloablative chemotherapy compound busulfan. Histological and *in vivo* imaging showing gradual donor macrophage engraftment of the brain between 6 and 24 weeks post-HSCT. Imaging the same macrophages over multiple months showed they became resident and had similar surveillant processes, as compared to the microglia they replaced. Half the host microglia were depleted by 2 weeks post-HSCT, with their density plateauing, while the cells doubled their process coverage area. Adult neurogenesis in both the sub-ventricular zone and hippocampus was completely ablated within 1 week, with no recovery up to 24 weeks post-HSCT. At 2 weeks post-HSCT, lamin B1 expression was lost with concurrent high expression of SA-beta-galactosidase, indicating cellular senescence. Further, microglia expressed the senescence/anti-apoptotic marker p21, were incapable of incorporating DNA as shown with 5-ethynyl-2'-deoxyuridine labeling, and expressed pospho-gamma-H2A.X, indicating DNA repair mechanisms. We administered the colony stimulating factor-1 antagonist PLX3397 to deplete host microglia, followed by withdrawal of PLX3397, while *In vivo* 2-photon imaging allow single-cell tracking of their recovery. In control mice, 4 weeks of PLX3397 caused 85% microglia depletion and drug withdrawal caused a 90% recovery within 4 days due to microglia proliferation. In HSCT mice, no host microglia recovered after PLX3397 depletion and withdrawal, yet donor macrophages showed robust proliferation in the brain at 5 days and beyond withdrawal, populating and tiling 95% of the cortex. In all HSCT brains, no host microglia cell division events were observed across multiple days of recovery, confirming their senescent state with busulfan treatment. Our results suggest busulfan DNA crosslinking causes microglia cell cycle arrest, with the induction of senescence along an anti-apoptotic p21 pathway. After HSCT, the gradual loss of microglia, due to their inability to proliferate, causes a minimal microglia density threshold, thereby allowing the permissive engraftment of macrophages in the brain. These results show for the first time the strong effect of busulfan induced microglia senescence and provide a therapeutic strategy for future cell transplantation-based brain gene therapies.

Disclosures: K. Sailor: 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.; Bluebird bio. **G. Agoranos:** A. Employment/Salary (full or part-time):; Bluebird Bio. **S. López-Manzaneda:** A. Employment/Salary (full or part-time):; Bluebird Bio. **S. Tada:** None. **B. Gillet-Legrand:** A. Employment/Salary (full or part-time):; Bluebird bio. **C. Guerinot:** None. **J.**

Masson: None. **C.L. Vestergaard:** None. **M. Bonner:** A. Employment/Salary (full or part-time):; Bluebird Bio. **K. Gagnidze:** A. Employment/Salary (full or part-time):; Bluebird Bio. **G. Veres:** A. Employment/Salary (full or part-time):; Biomarin Pharmaceutical Inc. **P. Lledo:** 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.; Bluebird Bio. **N. Cartier:** 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.; Bluebird Bio.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.09

Topic: B.11. Glial Mechanisms

Support: Doctoral Fellowship 303606
DGAPA UNAM PAPIIT IN204519
CONACYT CB-255399

Title: Postnatal exposure to lipopolysaccharide combined with high-fat diet consumption induces immune tolerance whitout protection in spatial working memory

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Abstract: High-fat diet (HFD) consumption has been related to metabolic alterations, such as obesity and cardiovascular problems, and has pronounced effects on brain plasticity and memory impairment. HFD exposure has a proinflammatory effect associated with microglial cell modifications in the hippocampus, a region involved in the working memory process. However, to our knowledge, few studies using male mice have evaluated the effect of HFD consumption after exposure to lipopolysaccharide (LPS) in the perinatal period on spatial working memory and the morphological changes in microglial cells. In the present study, male mice were injected with LPS (100 μg.kg-1 body weight) on postnatal day 3 and fed an HFD for 16 weeks after weaning. Ours results indicated that HFD consumption increased body weight gain, glucose

blood concentration and microglia process (number and length) from the hilus of the dentate gyrus, as well as spatial working memory impairment in comparison to control group. Interestingly, postnatal exposure to LPS in the early developmental stage combined with HFD consumption prevented glucose blood concentration increase, mRNA expression from pro-inflammatory cytokines (TNF-α and IL-1β) and microglial process with IL-10 mRNA expression increase in hippocampus, without changes in body weight gain and spatial working memory with respect HFD group. These findings indicate HFD consumption after postnatal LPS exposure induces immune tolerance, without prevention in spatial working memory impairment on male mice. We thank A. Aguilar, A. Castilla, N. Hernandez, D. Gasca, J. Gonzalez, and Michael Conrad Jeziorski for technical assitence.

Disclosures: I. Vargas-Rodríguez: None. **G. Pacheco-López:** None. **L.A. Reyes-Castro:** None. **E. Zambrano:** None. **A. Díaz-Ruíz:** None. **S. Diaz-Cintra:** None.

Poster

P312. Microglia: Disease Mechanisms I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P312.10

Topic: B.11. Glial Mechanisms

Support: NIH Grant AI073693

Title: Protective role of bradykinin in neuropsychiatric lupus

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Abstract: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the presence of anti-nuclear antibodies as well as a broad array of clinical manifestations affecting nearly every organ system. Neuropsychiatric syndromes affect over one half of SLE patients, presenting most commonly as headache, mood disorders and/or cognitive dysfunction. The pathophysiology of neuropsychiatric lupus has not been fully elucidated; however, it is likely triggered by cytokines such as HMGB1 and IFNa, as well as antibodies such as anti-NMDA receptor and anti-ribosomal P antibodies, passing through the blood-brain barrier and interacting with cells of the brain. We have developed a mouse model to study the effects of anti-dsDNA antibodies that cross-react with the NMDA receptor, termed DNRAbs, which are present in approximately 30% of SLE patients and whose presence in the CSF is associated with non-focal CNS manifestations of neuropsychiatric lupus.Using this model, we have previously demonstrated microglial activation, C1q-dependent dendritic pruning, enlarged place fields and behavioral deficits, which can be reversed by treatment with blood brain barrier – permeable

ACE inhibitors such as captopril. These findings suggest a therapeutic target for the treatment of neuropsychiatric lupus. However, due to the diverse role of ACE in a biologic system, it is yet to be determined how, specifically, ACE inhibitors work in this model. Whereas ACE is primarily known for its role in the renin-angiotensin system, it also plays an important role in the degradation of bradykinin.Our preliminary data suggest that bradykinin mediates the protective effects of ACE inhibitors. Furthermore, we believe this effect involves reversal of a pro-inflammatory microglial phenotype characterized in part by increased secretion of HMGB1. HMGB1 levels are elevated in serum of patients with SLE, and serum levels correlate with disease activity. In our neuropsychiatric lupus model, it has been shown that HMGB1 induces dendritic pruning by bridging NMDA receptors to C1q. In our preliminary studies with primary microglia cultures, we have shown that bradykinin treatment suppresses HMGB1 secretion. As a small peptide with numerous cleavage sites, bradykinin is rapidly degraded in serum. Therefore, to test our hypothesis in vivo, we have acquired 12 bradykinin analogues which have higher stability in plasma than bradykinin itself. Based on our preliminary data, we believe these analogues may reverse the neuropsychiatric phenotype of DNRAb+ mice. Our aim is to use these analogues to investigate bradykinin as a viable treatment target for neuropsychiatric lupus.

Disclosures: M. Mizrachi: None. **B. Diamond:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2011PG-MED011, Leona M. & Harry B. Helmsley Charitable Trust
ES_BI-2017, Christopher and Dana Reeve Foundation
EVPRI, University of Louisville Foundation

Title: Brain-spinal neuromodulation to improve stepping in individuals with motor complete spinal cord injury

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Abstract: Individuals diagnosed with motor complete spinal cord injury (SCI) have the capacity to recover walking in limited cases (Angeli2018, Gill2018). We have shown that spinal cord epidural stimulation (scES) combined with task specific training improved standing and stepping ability in individuals with SCI. The aim of this study is to understand mechanisms of combined

cervical spinal cord Transcutaneous Stimulation (scTS) with lumbosacral spinal cord Epidural Stimulation (scES) during non-weight bearing and weight-bearing stepping in individuals with motor complete SCI. Four individuals with complete SCI were implanted with an epidural electrode array and neurostimulator. Two individuals had received locomotor training with scES prior to the assessments and two individuals had never received locomotor training with scES. For the latter, step-scES mapping was performed prior to any assessment with the combination of cervical scTS+ lumbar scES. We collected electromyography of 6 lower extremity muscles bilaterally and performed kinematic analysis lower extremity joints during stepping in a gravity neutral device (GND) and on a treadmill with body weight support (BWST). While in the GND, individuals were asked to perform step like movements while in the presence of scES, scTS and scES+scTS. The same combinations were also assessed during stepping on BWST and manual assistance as needed. Peak to peak (PtoP) motor evoked potential generated during 2Hz scES was differently modulated during scES+scTS at C3, C5 and both sites combined. In most cases scTS at C3+C5 produced the greatest reduction in the PtoP amplitude when compared to scES alone and scES+scTS at either C3 or C5. When evaluating the integrated EMG of the tibialis anterior during stepping in the GND, a clear modulation is present when comparing scES with scES+scTS. The combination of scES+scTS at C3 and C5 sites reduces the relative difference between left and right activation promoting a more symmetrical pattern. Modulation was also present during stepping with BWST, resulting in improved coordination between flexors and extensors during the gait cycle when scES+scTS at C3 and C5 sites was applied compared to scES alone or scES+scTS at a single site. These results provide evidence the scES modulates network excitability of the injured spinal cord to allow for the integration of afferent and supraspinal descending input to generate stepping patterns. In addition, scTS applied to the cervical spine at or above the level of injury facilitate brain-spinal connectome as well as in addition to scES further modulates spinal excitability improving coordination during intentional stepping.

Disclosures: C. Angeli: None. **Y.P. Gerasimenko:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Christopher and Dana Reeve Foundation

Title: Cardiovascular function is modulated by lumbosacral spinal networks independent from other functions

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Abstract: Individuals with chronic cervical spinal cord injury (SCI) experience blood pressure instability that manifests as persistent hypotension, orthostatic hypotension, and autonomic dysreflexia. This dramatically decreases quality of life and increases cardiovascular morbidity and mortality, but clinically available treatments involve temporary symptom management rather than recovery and restoration of function.
We hypothesized that targeting integrated networks may reinstate cardiovascular regulatory mechanisms, utilizing lumbosacral spinal networks when supraspinal sympathetic input was chronically compromised. In this study, we anatomically, neurophysiologically, and pharmacologically identify specific spinal and peripheral circuitry responsible for cardiovascular stability using neuromodulation in 12 individuals with chronic cervical SCI implanted with a Medtronic 5-6-5 electrode array and neurostimulator. Blood pressure instability was evident in all individuals with SCI when monitored over six hours during sitting and during a 70-degree, head-up tilt test. However, only spinal cord epidural stimulation (scES) parameters targeted for cardiovascular function (CV-scES) significantly stabilized systolic blood pressure over six hours and during head-up tilt; non-cardiovascular (Non-CV-scES) configurations did not. When using high-resolution MRI to reconstruct the lumbosacral spinal cord and functional mapping, it was determined that CV-scES configurations that stabilized blood pressure were located over spinal segments L3, L4, and L5. Administration of Tamulosin, a selective $alpha_{1A}$ receptor antagonist, interferes with the ability of CV-scES to stabilize systolic blood pressure during sitting and during head-up tilt, indicating the role of sympathetic receptors on vascular smooth muscle. These results demonstrate neuromodulation specific to the lumbar spinal cord stabilizes blood pressure in chronic human SCI. Integrated systematic neuromodulation at the lumbosacral spinal has now demonstrated the ability to improve both motor and autonomic function - devastating consequences after severe SCI - while utilizing the same epidural implantation method, providing a potential multi-modality clinical treatment.

Disclosures: B. Ditterline: None. **G.F. Forrest:** None. **S. Mesbah:** None. **S. Kirshblum:** None. **J.M. Wecht:** None. **C.A. Angeli:** None. **S. Wang:** None. **S.J. Harkema:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: The Department of Defense W81XWH-19-1-0734
The Christopher & Dana Reeve Foundation ES_BI-2017

Title: Impact of lumbosacral epidural stimulation on bowel function in persons with spinal cord injury

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Abstract: Complications with bowel storage and evacuation are highly prevalent after spinal cord injury (SCI) and include dysmotility, chronic constipation and difficulty with evacuation, unexpected episodes of fecal incontinence, as well as autonomic dysreflexia, an abrupt rise in blood pressure associated with rectal distention and the passage of stool. Current management of neurogenic bowel dysfunction (NBD) includes optimizing stool consistency with adequate fiber and fluid intake and promoting regularly scheduled bowel emptying, primarily via mechanical rectal stimulation. However, large amounts of time are devoted to bowel programs and many individuals are also dependent on a caregiver for assistance. Epidural stimulation of the lumbosacral spinal cord (scES) has shown effectiveness in activating latent neural circuits with an ability to recover significant gains in autonomic function. The objective of the current study was to determine if scES was neuromodulatory for bowel function. Ten participants (7 males, 3 females) with motor complete chronic cervical SCI (C3-C8) completed anorectal manometry, wireless motility assessments, and questionnaires for bowel function before and after 80- (post-training 1) and 160-sessions (post-training 2) of training with scES (Cardiovascular-scES or Voluntary-scES, with and without Stand-scES). Anorectal manometry results revealed comparable resting and squeeze pressure values at baseline. By post-training 2, squeeze pressure significantly increased relative to baseline ($p < 0.05$). Rectal sensations improved at each post-training time point, occurring at a lower distention volume. Delays in upper gastrointestinal motility improved (reduced) at both post-training time points and a 50% reduction in the percentage of participants having severe NBD was evident by post-training 2. Taken together, these results indicate that scES can be used to improve anorectal dysfunction following chronic SCI, including an awareness of bowel sensations and the ability to generate sufficient squeeze pressure necessary for preventing fecal incontinence. Improved motility can reduce the occurrence of abdominal discomfort and the time needed for evacuation procedures. Even though scES was not directly configured for bowel, nor was stimulation ‘‘on’’ during assessments, sufficient excitation of neural networks, comprised of overlapping motor and autonomic networks, during training may have led to improved adaptations in internal/external anal sphincter dynamics important for storage and evacuation reflexes. Post-SCI motility changes may also involve alterations in the intact vagal neurocircuitry.

Disclosures: S.C. Aslan: None. **B. Ugiliweneza:** None. **D. Wang:** None. **E. Wyles:** None. **K. Johnson:** None. **T. Abell:** None. **C.H. Hubscher:** None. **S.J. Harkema:** None. **A.N. Herrity:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2016PG-MED001, Leona M. & Harry B. Helmsley Charitable Trust
ES_BI-2017, Christopher and Dana Reeve Foundation

Title: Lumbosacral spinal cord epidural stimulation acutely improves cerebral hemodynamics during orthostatic stress in individuals with chronic spinal cord injury

Authors: S. WANG[1,2], J. M. WECHT[3,4], B. DITTERLINE[1,2], S. MESBAH[1], G. F. FORREST[5,6], O. BLOOM[7,8], A. V. OVECHKIN[1,2], S. J. HARKEMA[1,2,9], J. D. GUEST[10]; [1]Kentucky Spinal Cord Injury Res. Ctr., [2]Neurolog. Surgery, Sch. of Med., Univ. of Louisville, Louisville, KY; [3]James J Peters VA Med. Ctr., Bronx, NY; [4]Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Kessler Fndn., West Orange, NJ; [6]Physical Med. & Rehabilitation, Rutgers New Jersey Med. Sch., Rutgers, the State Univ. of New Jersey, Newark, NJ; [7]The Feinstein Inst. For Med. Res., Manhasset, NY; [8]Zucker Sch. of Med. at Hofstra Northwell, Hempstead, NY; [9]Frazier Rehab Inst., Louisville, KY; [10]Neurolog. Surgery/Miami Project to Cure Paralysis, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Introduction: Spinal cord injury (SCI) results in cardiovascular dysfunctions, including orthostatic hypotension (OH), defined as a substantial drop in arterial blood pressure (BP) when assuming an upright position. OH may cause cerebral hypo-perfusion possibly linked to a chronic decline of cognitive function, and to a loss of normal ability of cerebral vessels to buffer BP oscillations potentially damaging this critical vasculature and increasing risk of stroke. OH and cerebral hypo-perfusion are difficult to manage, adversely impacting health, functional rehabilitation and quality of life. We previously showed that lumbosacral spinal cord epidural stimulation (scES), optimized for cardiovascular function (CV-scES) mitigated OH in individuals with chronic SCI. This study evaluated the acute effects of CV-scES on cerebral blood flow velocity and cerebrovascular buffering of BP oscillations in participants with chronic SCI. **Methods:** Ten individuals with chronic cervical SCI and severe cardiovascular dysfunction had a 16-electrode array implanted over the lumbosacral spinal segments. Personalized neuromodulation was comprised of specific cohorts (anode and cathode electrodes, amplitude, frequency, and pulse width selection) interleaved for a CV-scES specific configuration of parameters designed to maintain systolic BP between 110-120 mmHg and to avoid inducing lower extremity muscle activity. Orthostatic tolerance was tested with a 70° head-up tilt maneuver lasting up to 30 minutes, with and without CV-scES. Subjective pre-syncopal symptom severity was recorded using a 0-10 scale. Beat-to-beat blood flow velocity at the

middle cerebral artery (mCBFv) and mean arterial pressure (MAP) at the finger were monitored simultaneously. Sympathetically mediated cerebrovascular buffering of BP oscillations was estimated using wavelet derived phase synchronization index between mCBFv and MAP. **Results:** Compared to tilt without stimulation, CV-scES increased the tolerated tilt time (10 ± 3 vs. 30 ± 0 minutes, respectively, p=0.0003), reduced symptom severity (5.5 ± 0.9 vs. 0.2 ± 0.1, p=0.0002), reduced the fall in MAP (-28 ± 4 vs. -2 ± 4 mmHg, p=0.001), reduced the fall in mCBFv (-16 ± 2 vs. -8 ± 1 cm/s, p=0.001 and improved cerebrovascular buffering of BP oscillations indicated by reduced phase synchronization index within the ultra-low frequency range during tilt (0.64 ± 0.22 vs. 0.42 ± 0.14, p=0.05). **Conclusions:** Maintenance of BP with acute CV-scES during orthostatic stress in individuals with chronic cervical SCI is associated with improved cerebral hemodynamics and buffering of BP oscillations resulting in prolonged orthostatic tolerance.

Disclosures: S. Wang: None. **J.M. Wecht:** None. **B. Ditterline:** None. **S. Mesbah:** None. **G.F. Forrest:** None. **O. Bloom:** None. **A.V. Ovechkin:** None. **S.J. Harkema:** None. **J.D. Guest:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Christopher and Dana Reeve Foundation

Title: Improved cognition and cerebral hemodynamics after lumbosacral spinal cord epidural stimulation intervention in individuals with chronic spinal cord injury

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Abstract: Due to impaired sympathetic control of cardiovascular regulation, individuals with chronic cervical and upper thoracic spinal cord injury (SCI) exhibit decreased resting blood pressure when compared with non-injured individuals. Hypotension has been associated with cognitive impairment in the non-injured population. Poorer cognitive performance on cognitive tasks has been reported in hypotensive individuals with SCI compared to normotensive individuals with SCI, which was related to inadequate blood pressure and cerebral blood flow

velocity responses during testing. Impaired cognitive performance after SCI impacts multiple domains, including attention and concentration, memory, abstract reasoning, verbal learning, and information processing, which delays rehabilitation and social integration and ultimately decreases quality of life and independence. However, there are no clinically available therapies to restore the underlying sympathetic deficit, and efforts to pharmacologically support blood pressure to mitigate cognitive deficits are not consistently successful. Physical exercise can prevent age-related loss of brain tissue in non-injured individuals and leads to gray and white matter growth in a variety of neural structures associated with cognition including the hippocampus and frontal, temporal, and parietal cortices. We investigated the cognitive and cerebrovascular effects of an epidural stimulation intervention to support blood pressure combined with physical exercise. Five individuals with chronic cervical SCI, implanted with a Medtronic 5-6-5 paddle array over the lumbosacral spinal cord, participated in this study. Spinal cord epidural stimulation (scES) was used 1) to target cardiovascular regulation (CV-scES) and maintain systolic blood pressure between 110-120 mmHg while sitting and 2) to facilitate standing overground (Stand-scES). Individuals participated in at least 80 daily sessions of CV-scES (6 hours) followed by Stand-scES (2 hours). Cognitive function in the domains of learning, memory, attention, and executive function domains were examined pre- and post-Intervention with simultaneous recording of cerebral blood flow velocity without active scES. Post-intervention, we found improved cognitive flexibility, verbal fluency, memory and attention which were associated with increased cerebral blood flow velocity in the middle and posterior cerebral arteries. These data suggest that targeted CV-scES combined with Stand-scES may lead to beneficial adaptations in neurovascular coupling, which has the potential to improve quality of life and independence of individuals with chronic SCI.

Disclosures: B. Ditterline: None. **M. Cotter:** None. **M. Luttrell Greenwell:** None. **E. Cash:** None. **J. Fisher:** None. **S. Wang:** None. **E. Rejc:** None. **S.J. Harkema:** None. **J. Wecht:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: The National Institutes of Health Roadmap Initiative, Office of Strategic Coordination – The Common Fund, Other Transactions, Stimulating Peripheral Activity to Relieve Conditions (SPARC) Program Award # OT2OD024898

Title: Epidural stimulation for lower urinary tract function in rats with spinal cord contusion injuries

Authors: *N. L. WILKINS[1], R. F. HOEY[1], D. MEDINA-AGUIÑAGA[1], F. KHALIFA[3], B. UGILIWENEZA[2], J. FELL[1], A. NAGLAH[3], A. S. EL-BAZ[3], A. N. HERRITY[2], S. J. HARKEMA[2], C. H. HUBSCHER[1];
[1]Anatom. Sci. and Neurobio., [2]Neurolog. Surgery, Univ. of Louisville Sch. of Med., Louisville, KY; [3]Bioengineering, Univ. of Louisville J. B. Sch. of Engin., Louisville, KY

Abstract: Epidural stimulation for lower urinary tract function in rats with spinal cord contusion injuries Natasha Wilkins, Robert F. Hoey, Daniel Medina-Aguiñaga, Fahmi Khalifa, Beatrice Ugiliweneza, Jason Fell, Ahmed Naglah, Ayman S. El-Baz, April N. Herrity, Susan J. Harkema, Charles H. Hubscher Abstract:Spinal cord injury (SCI) can have long and life-changing effects on lower urinary tract (LUT) function. Epidural stimulation of the lumbosacral spinal cord (scES) has demonstrated clinical effectiveness for improving both bladder compliance and reflex control of voiding. Our previous pre-clinical scES mapping studies at T13-L2, L3-4, and L5-S1 in spinally intact and T9-transected male and female rats further demonstrate efficacy of scES for modulating bladder storage and emptying. In the current study, maximally effective scES for maintaining continence or for reflex voiding was applied at the same three spinal levels with two modifications: 1) female rats with incomplete T9 level injuries of different severities were examined (clinically relevant spinal contusion model); and 2) a novel miniature 15-electrode array was used to enhance specificity at each spinal cord location. The results obtained during bladder fill and void cycles conducted under urethane anesthesia indicate frequency and intensity dependent effects on void volume, inter-void interval, detrusor contraction, and external urethral sphincter (EUS) activation. Responsiveness of the LUT to scES differed based upon severity of injury, whereby contusion injuries displayed some responses similar to complete transected animals and some that were similar to spinally intact animals. Some scES cohorts, for example, improved bladder compliance, producing a low-pressure fill of the bladder for a prolonged storage period with L1 level stimulation whereas others resulted improved voiding efficiency, resulting in timed activation of the detrusor triggering a void response (L6 level stimulation). The current study's novel electrode design with improved selectivity permitted enhanced LUT function at much lower stimulus intensities without any concomitant lower hindlimb movements. The potential for selective activation of the LUT circuitry at multiple levels with differing injury severities adds to an accumulating body of evidence regarding the use of scES as a singular SCI device intervention that can programmed to target multiple systems simultaneously.

Disclosures: N.L. Wilkins: None. **R.F. Hoey:** None. **D. Medina-Aguiñaga:** None. **F. Khalifa:** None. **B. Ugiliweneza:** None. **J. Fell:** None. **A. Naglah:** None. **A.S. El-Baz:** None. **A.N. Herrity:** None. **S.J. Harkema:** None. **C.H. Hubscher:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2011PG-MED011, Leona M. & Harry B. Helmsley Charitable Trust
ES_BI-2017, Christopher and Dana Reeve Foundation
1OT2OD024898, National Institutes of Health
ES2-CHN-2013, Craig H. Neilsen Foundation, Christopher and Dana Reeve Foundation
2011PG-MED011, Leona M. & Harry B. Helmsley Charitable Trust
ES1-2011, Christopher and Dana Reeve Foundation
1R01EB007615, National Institutes of Health

Title: Selection of lumbosacral spinal cord epidural stimulation parameters for motor and autonomic functions in persons with chronic spinal cord injury

Authors: *S. HARKEMA[1,4,2], E. REJC[1,2], A. N. HERRITY[1,2], C. HUBSCHER[5,1], G. F. FORREST[6,7], S. MESBAH[1], M. BOAKYE[1,2], C. A. ANGELI[1,4,3];
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Abstract: Lumbosacral spinal cord epidural stimulation (scES) has shown effectiveness for the recovery of walking, standing, voluntary movement, cardiovascular regulation and for the restoration of voluntary and reflex control of the lower urinary tract in individuals with motor complete spinal cord injury (SCI). The lack of clear guidelines on stimulation parameters is perceived as a significant barrier toward the use of scES for functional improvement by 83% of the US physicians who participated in a dedicated survey (Solinsky et al., 2019). To date, the major challenges related to the selection of scES parameters for promoting motor and autonomic functional recovery in severe SCI individuals are that i) these parameters are task- and individual-specific; and ii) the combination of stimulation parameters potentially available is vast, as exemplified by the over 43 million different electrode configurations potentially available using a 16-electrode array. The objective of this study is to demonstrate multi-functional mapping based on personalized neuromodulation with cohorts (anode and cathode selection, pulse width, frequency, and amplitude) resulting in specific scES configurations targeting differentially motor, cardiovascular and urinary functions. Our approach combines 3D reconstruction using high resolution MRI with spatial-temporal and functional mapping for targeted motor or autonomic responses in research participants implanted with a Medtronic 5-6-5 neurostimulator and electrode array placed over lumbosacral spinal segments. Electromyographic (EMG) activity and movement, continuous measurements of blood pressure and heart rate using a non-invasive blood pressure monitoring system and/or cystometrograms were recorded depending on the functional mapping session. Data collected from thirty-two research participants with chronic SCI identified mapping configurations that were selectively effective for motor, cardiovascular and/or bladder function. Initial functional mapping studies

resulted in voluntary movements in 32/32 participants, cardiovascular regulation in 22/22 participants, and urodynamic improvements in capacity and voiding in 6/6 participants. Importantly, these results reveal the complex dynamics and interplay between motor and autonomic systems that is being integrated and regulated within the lumbosacral spinal cord below the level of SCI in humans.

Disclosures: S. Harkema: None. **E. Rejc:** None. **A.N. Herrity:** None. **C. Hubscher:** None. **G.F. Forrest:** None. **S. Mesbah:** None. **M. Boakye:** None. **C.A. Angeli:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SCIRB Grant C31290GG
Christopher and Dana Reeve Foundation

Title: Robotic postural stand training with epidural stimulation improved reactive standing postural control in individuals with motor complete SCI

Authors: *C. BOWERSOCK[1], T. PISOLKAR[1], I. OMOFUMA[2], T. LUNA[2], M. KHAN[2], V. SANTAMARIA[2], C. ANGELI[1], J. STEIN[2], S. AGRAWAL[2], S. HARKEMA[1], E. REJC[1]; [1]Univ. of Louisville, Louisville, KY; [2]Columbia Univ., New York, NY

Abstract: With spinal cord epidural stimulation (scES) and activity-based training, individuals with motor complete spinal cord injury (SCI) can recover the ability to stand with independent lower limb extension by grasping onto handlebars for balance assistance. However, standing while using the upper limbs for balance assistance alters postural control strategies and limits peripheral sensory information received by the lumbosacral spinal circuitry. Enhanced rehabilitation strategies are needed to train a more functional standing environment.
The aim of this study was to determine the effect of robotic postural stand training on reactive standing postural control without the use of the upper limbs for balance assistance in motor complete SCI individuals implanted with scES. Three individuals with chronic sensory and motor complete SCI (C4 (n=2), C3 (n=1)) were recruited. Participants previously received stand training with scES using regular assistive devices for balance assistance, achieving independent knee extension.
The robotic upright stand trainer (RobUST) used in this study contains an aluminum frame with 8 motors, which control the forces applied at the trunk and pelvis by cables attached to harnesses. During the experimental sessions, the RobUST elicited lateral trunk perturbations at a participant-specific magnitude aimed at achieving ~50% successful control before training. A

constant force applied by the RobUST assisted the pelvis. For each perturbation, participants attempted to maintain standing posture without use of the handlebars. Attempts were successful if the participant maintained or regained standing posture after the perturbation without using the handlebars and without additional assistance provided by a trainer. Successful perturbation control, vertical ground reaction force (Vgrf), and lower limb EMG response were quantified for each attempt. Participants performed ~80 postural stand training sessions with the RobUST and scES, which included perturbation, stable stand, and self-initiated trunk and arm movement. Assessments were conducted pre, mid, and post training.
Successful control of lateral trunk perturbations improved from 50% pre training to 85% after training. This was associated with a 450% increase in ipsilateral lower limb EMG activity and 19% increase in ipsilateral Vgrf in response to the lateral perturbations after training. This study shows that individuals with motor complete SCI and scES can improve reactive standing postural control without the use of the upper limbs, and this improvement is associated to lower limb EMG and kinetic adaptations promoted by robotic postural stand training.

Disclosures: C. Bowersock: None. **T. Pisolkar:** None. **I. Omofuma:** None. **T. Luna:** None. **M. Khan:** None. **V. Santamaria:** None. **C. Angeli:** None. **J. Stein:** None. **S. Agrawal:** None. **S. Harkema:** None. **E. Rejc:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ES_BI-2017, Christopher and Dana Reeve Foundation, Leona M. & Harry B. Helmsley Charitable Trust
2016PG-MED001, Leona M. & Harry B. Helmsley Charitable Trust
ES2-CHN-2013, Craig H. Neilsen Foundation
UofL Health - University of Louisville Hospital and Medtronic Plc

Title: Neuroanatomical mapping of the spinal cord lumbosacral enlargement in individuals with chronic spinal cord injury

Authors: *S. MESBAH[1], A. N. HERRITY[2], I. CHOPRA[1], B. UGILIWENEZA[2], C. A. ANGELI[3], Y. GERASIMENKO[4], M. BOAKYE[2], S. J. HARKEMA[1];
[1]Kentucky Spinal Cord Injury Res. Ctr., [2]Neurosurg., [3]Bioengineering, [4]Physiol. and Biophysics, Univ. of Louisville, Louisville, KY

Abstract: Spinal cord injury (SCI) caused by acute physical trauma often results in spinal cord lesions at the site of injury, while the rest of the spinal cord remains fairly intact. Damage to the

spinal cord is complex and can affect the body with varying degrees of sensorimotor and autonomic dysfunction. The use of spinal stimulation, with electrodes placed epidurally, induces a subthreshold electric field sufficient to increase the excitability of relevant neuronal circuits in the intact portion of the lumbosacral enlargement that is capable of driving not only voluntary activity, but also restoring multi-system autonomic improvements in individuals with chronic SCI. However, there is a lack of information regarding whether various neuroanatomical factors in the intact portion of the spinal cord may play an important role in functional recovery with the application of spinal cord epidural stimulation (scES). Our recent study showed that maximizing the spinal cord lumbosacral coverage by the scES paddle electrode may lead to better functional outcomes.The goal of this study is to use MRI-based spinal cord 3D modeling and quantitative analysis to gain a deeper understanding of the neuroanatomical substrates of the spinal cord and the functional properties of the excitable tissue. Twelve individuals with clinically diagnosed chronic, motor complete SCI that were implanted with scES were included (age: 34±10.9, gender: 10 male, 2 female, time since injury: 8.2±9.9, AIS: 6 A, 6 B). High resolution MRI scans of spinal cord were recorded prior to the implant surgery. The results show that the size of the spinal cord and nerve roots, the conus tip and the relationship between the spinal cord levels and vertebral levels, particularly at lumbosacral, is variable across individuals. Volumetric coverage of the lumbosacral spinal cord ranges from 33.4% to 90.4% across participants. The location and position of the excitable tissue (spinal cord and the nerve roots) with respect to the electrode contacts varies across individuals and impacts the patterns of neurophysiological responses. Distinct regions of the spinal cord at the lumbosacral enlargement control certain muscle recruitment patterns in response to scES. Finally, using MRI-based spinal cord modeling as a guide for prediction of optimum scES paddle placement prior to the implant surgery has substantially reduced the duration of the electrophysiological mapping and number of scES placements tested in the surgery. These findings highlight the crucial role that neuroanatomical characteristics of the spinal cord specific to each individual plays in reaching maximum functional benefits with scES.

Disclosures: S. Mesbah: None. **A.N. Herrity:** None. **I. Chopra:** None. **B. Ugiliweneza:** None. **C.A. Angeli:** None. **Y. Gerasimenko:** None. **M. Boakye:** None. **S.J. Harkema:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ES_BI-2017, Christopher and Dana Reeve Foundation

Title: The effects of core-specific and non-specific training with epidural stimulation on trunk kinematics of individuals with chronic motor complete spinal cord injury.

Authors: *K. JOSHI[1], N. SMITH[2], E. REJC[3], S. J. HARKEMA[4,6], C. ANGELI[5,6];
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Abstract: Past evidence suggests that spinal cord epidural stimulation (scES) in individuals with spinal cord injury (SCI) as well as core-based training lead to improvement in trunk stability in activities such as tall-sit, reaching, or leaning (Gill et al., 2020). Effects of non-specific training, however, have not been explored much. The aim of this study is to compare the effects of core-based and non-core based training with scES on trunk kinematics.17 individuals with chronic motor complete SCI (Age: 35.3 ± 12.7 yrs; time post-injury: 8.8 ± 8.4 yrs) were implanted with a scES unit and were randomized into four intervention groups (Voluntary – Vol, Cardiovascular – CV, Vol with stand, and CV with stand). Vol intervention comprised tasks to improve core stability. CV intervention comprised stabilizing BP without causing any muscle activity. Each group performed two 80-session sets. Standing was added to Vol and CV groups in the second intervention set. Data was collected at 3 time-points (Post Implant, Post Int1, and Post Int2) and each individual was asked to perform core activities without and with Vol-scES. Full-body kinematics was acquired and later processed for tall sitting, forward/back lean (FB), and lateral lean (LR). Center of mass of pelvis and torso was then used to calculate anterior-posterior (AP) and lateral torso displacement along with torso velocity in all four directions. Individuals were classified as non-responders if the reduction in all velocities was < ± 10% with scES. Responders were further divided into positive, negative, and neither (multidirectional) based on how their displacements and velocities changed. For sitting, decreased displacements and velocities were the main goal. For FB & LR movements, the goal was to have the relative change in displacement be better than the change in velocity in the direction of movement, and have decreased velocities and displacements in the other direction.Responder distribution for stim conditions for all three activities showed that both Vol group and CV group responded to the two interventions. In Sitting and LR, Vol group had more positive responders at Int1 and Int2, whereas CV group had more negative and non-responders. In FB, Vol group did not have as many positive responders, however the responder distribution Post Int1 and Int2 were similar. In contrast, CV group had more positive responders at Int2. These results indicated that Vol intervention along with scES leads to improved trunk stability and control in core-specific tasks. Improvements seen in FB control in the CV group may have been influenced by stand training part of the combination training during Int2.

Disclosures: K. Joshi: None. **N. Smith:** None. **E. Rejc:** None. **S.J. Harkema:** None. **C. Angeli:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SCIRB Grant C31290GG
Christopher and Dana Reeve Foundation

Title: Robotic stand training and spinal cord epidural stimulation improved standing postural control in individuals with motor complete spinal cord injury

Authors: *E. REJC[1], T. PISOLKAR[1], C. BOWERSOCK[1], I. OMOFUMA[2], T. LUNA[2], M. KHAN[2], V. SANTAMARIA[2], C. ANGELI[1], J. STEIN[2], S. AGRAWAL[2], S. HARKEMA[1]; [1]Univ. of Louisville, Louisville, KY; [2]Columbia Univ., New York, NY

Abstract: Postural control is a foundational ability to generate effective motor outputs and is drastically impaired after spinal cord injury (SCI). In the last decade, proof of principle studies showed that spinal cord epidural stimulation (scES) and activity-based training can promote the recovery of standing and walking after motor complete SCI. However, participants' postural control was always self-assisted by their upper limbs placed on assistive devices (i.e. walker), or via body weight support systems. The aim of this study was to assess the effects of robotic postural stand training with scES in 3 individuals with chronic sensory and motor complete SCI (level of the lesion: cervical (C) 4 (n=2) or C3 (n=1)). These individuals had previously received extensive stand training with scES using regular assistive devices for self-balance assistance, achieving independent knees extension. The robotic upright stand trainer (RobUST) is a motion capture-based system that consists of an aluminum frame with 8 motors, which control the forces applied at the trunk and pelvis by cables attached to a harness. RobUST can: (*i*) provide a force field (FF) environment to assist the trunk as needed. Participants can move freely within the FF boundary, while a restoring force is applied when the trunk moves beyond the FF boundary. (*ii*) elicit postural perturbations. This allowed participants to train without the use of upper limbs for balance control (free hand). Participants performed ~80 sessions of RobUST postural training with scES (5 days/week; 1 hour per session). Postural training included periods of stable standing, self-initiated trunk and arm movements, and trunk perturbations with free hands. Standing postural control was assessed prior to the beginning of training (Pre), and after ~40 (Mid) and ~80 (Post) training sessions. At Pre, only 1 individual was able to maintain consistently stable standing with free hands and No FF (i.e. outside of RobUST), and none of the participants could perform self-initiated trunk movements in the same standing condition. RobUST FF immediately enabled the practice of stable standing and trunk movements with free hands in all participants. After training, all individuals could maintain stable standing, generate controlled trunk movements and arm reaching movements while standing with free hands and No FF, receiving only external assistance for pelvis control. RobUST postural training with scES promoted remarkable standing postural control improvements, and showed potential to enhance the overall level of functional recovery after severe cervical SCI.

Disclosures: E. Rejc: None. **T. Pisolkar:** None. **C. Bowersock:** None. **I. Omofuma:** None. **T. Luna:** None. **M. Khan:** None. **V. Santamaria:** None. **C. Angeli:** None. **J. Stein:** None. **S. Agrawal:** None. **S. Harkema:** None.

Poster

P368. Spinal Cord Injury: Recovery III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P368.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ES_BI-2017, Christopher and Dana Reeve Foundation

Title: The effect of epidural stimulation targeted at trunk stability on trunk kinematics of individuals with chronic motor complete spinal cord injury.

Authors: *N. SMITH[1], K. JOSHI[2], E. REJC[3], S. J. HARKEMA[4,6], C. ANGELI[5,6];
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Abstract: Past evidence suggests that spinal cord epidural stimulation (scES) in individuals with spinal cord injury (SCI) as well as core-based training programs lead to improvement in trunk stability in activities such as tall-sit, reaching, or leaning (Gill et al., 2020). The aim of this study is to compare the acute effects of scES prior to training and also explore the effects of non-core based training. 17 individuals with chronic motor complete SCI (Age: 35.3 ± 12.7 yrs; time post-injury: 8.8 ± 8.4 yrs) were implanted with a scES unit and were randomized into four intervention groups (Voluntary – Vol, Cardiovascular – CV, Vol with stand, and CV with stand). Vol intervention comprised tasks to improve core stability. CV intervention comprised stabilizing BP without causing any muscle activity. Data was collected at 2 time-points (Post Implant and Post Int1) and each individual was asked to perform core activities without and with Vol-scES. Full-body kinematics was acquired and later processed for tall sitting, forward/back lean (FB), and lateral lean (LR). Center of mass of pelvis and torso was then used to calculate anterior-posterior (AP) and lateral torso displacement along with torso velocity in all four directions. Individuals were classified as non-responders if the reduction in all velocities was < ± 10% with scES. Responders were further divided into positive, negative, and neither (multidirectional) based on how their displacements and velocities changed. For sitting, decreased displacements and velocities were the main goal. For FB & LR movements, the goal was to have the relative change in displacement be better than the change in velocity in the direction of movement, and have decreased velocities and displacements in the other direction.Responder distribution at post implant for Vol and CV groups were similar for all three

activities. The distribution of Post Int1 for CV and Post Implant for CV was same in FB, but different in sitting and LR. The contrasting results in LR may be attributed as a secondary effect of stand training in CV+Stand group. It can be inferred that without any training, scES has an immediate effect on trunk stability and control. Not all individuals had a positive improvement with scES, however there were not many non-responders. It was also evident that except for LR, the CV group responded in a similar way to Vol-scES at post implant and Post Int1 conditions.

Disclosures: N. Smith: None. **K. Joshi:** None. **E. Rejc:** None. **S.J. Harkema:** None. **C. Angeli:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.01

Topic: D.03. Somatosensation – Pain

Title: Contextual control of conditioned pain tolerance and endogenous analgesic systems

Authors: S. TRASK[1], J. S. MOGIL[2], F. J. HELMSTETTER[3], C. L. STUCKY[4], ***K. E. SADLER**[4];
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Abstract: The mechanisms underlying the transition from acute to chronic pain development are unclear, but likely involve the persistence or strengthening of pain memories. Environmental context was recently described as a critical regulator of pain memory; both rodents and humans exhibit increased pain sensitivity in environments recently associated with a single painful experience. It is unknown however, how repeated exposure to a painful stimulus (unconditioned stimulus; UCS) in a unique context (with distinct olfactory and visual cues) modifies pain expression in that environment. To answer this question, we paired one context with administration of a mild visceral pain stimulus (intraperitoneal injection of 0.9% acetic acid) and a similar, but distinct, context with a vehicle injection over the course of three days. On the fourth day, animals received either a vehicle injection in both the acid-trained and vehicle-trained chamber or a 0.9% acetic acid injection in both the acid-trained and vehicle-trained chamber. Similar to previous reports, male mice exhibited conditional hypersensitivity when given a vehicle injection in the acid-trained environment on day 4, but female mice did not. However, when given an acetic acid injection on day 4, both male and female mice exhibited context-dependent pain tolerance in the acid-trained environment. Neither male nor female mice exhibited UCS-induced mechanical hypersensitivity in the acid-trained context, and female mice exhibited less acid-induced abdominal writhing in the acid-trained context than the vehicle-

trained context, despite receiving identical acetic acid injections prior to being placed into each context. This observed pain tolerance was not explained by altered stress responses as circulating corticosterone levels were similar in the acid- and vehicle-trained environments on day 4. Systemic administration of naloxone immediately before UCS administration on day 4 completely blocked the context-dependent conditioned analgesia. These experiments suggest that endogenous opioid systems are engaged as a context-dependent conditioned compensatory response for painful stimuli. Successful determination of the brain circuits involved in the recruitment of this response may allow for the manipulation of pain memories which may be maladaptive in chronic pain states.

Disclosures: S. Trask: None. **J.S. Mogil:** None. **F.J. Helmstetter:** None. **C.L. Stucky:** None. **K.E. Sadler:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.02

Topic: D.03. Somatosensation – Pain

Title: Endogenous analgesic synergy between mu opioid receptor and CB_1 receptor constitutive activity prevents chronic postoperative pain with greater efficacy in female mice

Authors: *C. NORRIS[1], A. MARTEL MATOS[2], D. D. DOS SANTOS[1], D. E. SELLEY[3], L. J. SIM-SELLEY[4], B. K. TAYLOR[1];
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Abstract: The endocannabinoid system is a promising target for the treatment of chronic pain. We previously reported that μ-opioid receptor constitutive activity (MOR_{CA}) in the dorsal horn of the spinal cord (DH) maintains injury-induced latent pain sensitization (LS) in a state of remission. We hypothesized that pain remission is maintained by cannabinoid CB_1 receptor constitutive activity (CB_{1CA}) as well. C57BL/6 mice were subjected to either plantar skin/muscle incision or sham surgery. Mechanical sensitivity was tested with the application of von Frey filaments. After the resolution of mechanical hypersensitivity (21 days post-surgery), CB_1 inverse agonist AM281 (10μg) or vehicle was injected intrathecally. AM281 but not vehicle reinstated mechanical hypersensitivity in the incision but not sham groups. Dose response studies (10ng-30μg) revealed that AM281 was 100X more potent in female (ED50 4.3ng) versus male (ED50 4.3μg) mice. Next, we calculated equipotent dose ratios of AM281:CTOP (a MOR-selective inverse agonist) to be 4300:1 for males, and 4:1 for females and tested multiple doses of their combinations (10pg-10μg, i.t.). We found a 1000-fold leftward shift to the dose response

suggesting dramatic synergy. Notably, the AM281:CTOP combination was 100X more potent in female vs male mice. Next, we collected spinal cords at POD24-25, and conducted agonist-stimulated [^{35}S]GTPγ;;;S binding in isolated membranes. Injury did not change CB1 agonist (CP55,940)- or MOR agonist (DAMGO)-stimulated E_{max}, or EC_{50} values, indicating no change in G-protein activation. CP55,940+DAMGO (1:3) combination produced no stimulation greater than the predicted additive effects suggesting that synergy was occurring downstream of G-protein activation. We next studied glutamate (10s 300µM)-evoked Ca^{2+} mobilization in DH transverse slices, 21 days after incision or sham surgery. Pre-application of AM281 (5min 100µM) increased the duration of the glutamate-evoked Ca^{2+} response in female but not male mice. Previous literature suggested that the endocannabinoid system interacts with ryanodine-sensitive internal Ca^{2+} stores, so we combined AM281 with the ryanodine receptor blocker JTV519 (1µM), which blocked the effect if AM281. In summary, we report that: surgery establishes $CB1_{CA}$ that maintains LS in remission with 100X greater potency in females; in the setting of LS, AM281 exerts a sexually dimorphic effect on ryanodine-sensitive internal Ca^{2+}stores; and endogenous analgesic synergy between MOR_{CA} and $CB1_{CA}$ prevents chronic postoperative pain with 100X greater potency in females.

Disclosures: C. Norris: None. **A. Martel Matos:** None. **D.D. dos Santos:** None. **D.E. Selley:** None. **L.J. Sim-Selley:** None. **B.K. Taylor:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.03

Topic: D.03. Somatosensation – Pain

Support: R01NS112632

Title: S1pr1 agonism in the central amygdala reduces neuropathic pain

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Abstract: Sphingosine-1-phosphate receptors (S1PRs) are an emerging target for the treatment of persistent pain. An emerging literature reports that the S1PR1 agonist / functional antagonist fingolimod, an FDA-approved disease modifying agent for multiple sclerosis (MS), reduces pain-like behaviors in models of inflammatory and neuropathic pain. For example, we reported that systemic administration of fingolimod reduced mechanical allodynia in the experimental autoimmune encephalomyelitis (EAE) model of MS. This reduction in mechanical allodynia was mimicked by the S1PR1 agonist SEW2871 and blocked by the S1PR1 antagonist, W146,

indicating an S1PR1-dependent mechanism. However, the anatomical sites of action remains unclear. The central amygdala (CeA) is an important supraspinal center for the processing of nociceptive input and is an emerging target for chronic pain. Using fluorescence *in situ* hybridization, we found robust S1PR1 expression in the central nucleus of the amygdala (CeA), and so tested the hypothesis that microinfusion of S1PR1 agonists into the CeA would reduce neuropathic pain. Adult male C57BL/6 mice underwent spared nerve (SNI) at the left sciatic nerve, followed by guide cannula implantation into the right CeA. Mechanical allodynia was tested with the application of von Frey hairs (up/down method) to the plantar surface of the left hindpaw. SNI dramatically reduced mechanical threshold, peaking at 14 days post-surgery. At this timepoint, saline or S1PR1 agonist was administered into the CeA. We found that intra-CeA fingolimod (0.01ng; 300nL) or CYM5442 (0.6ng; 300nL) but not saline attenuated mechanical allodynia, with a peak effect at 10 minutes and lasting 30-45 minutes (n=5-7; 2-way RM ANOVA with Bonferroni post-test, $P< 0.05$). Additionally, we found that intra-CeA SEW2871 (0.1ug & 1.0ug; 0.2ul) but not saline attenuated mechanical but not cold allodynia with a peak effect at 15 minutes and lasting 30-45 minutes (n=8-9; 2-way RM ANOVA with Bonferroni post-test, $P<0.05$). We conclude that the CeA engages S1PR1 signaling to reduce mechanical allodynia in the SNI model of neuropathic pain. These results point to S1PR1s in the CeA as a target for future pharmacotherapy of neuropathic pain.

Disclosures: S.R. Lamerand: None. **A.H. Cooper:** None. **P.L. Sheets:** None. **B.K. Taylor:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.04

Topic: D.03. Somatosensation – Pain

Support: R01NS45954
R01NS62306
R01DA37621

Title: Tonic inhibition of mechanical and cold nociception and itch by neuropeptide Y Y2 receptors in primary afferent neurons

Authors: P. BASU[1], A. MADDULA[1], H. HERZOG[2], T. S. NELSON[1], ***B. K. TAYLOR**[1]; [1]Anesthesiol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Garvan Inst. of Med. Res., Darlinghurst, Australia

Abstract: Exogenous or endogenous neuropeptide Y (NPY) acts at its cognate Y1 receptor in the dorsal horn of the spinal cord to tonically inhibit behavioral and molecular signs of

inflammatory and neuropathic pain. The actions of NPY at the Y2 receptor (Y2R), the other Gi-coupled receptor that is highly expressed in the somatosensory nervous system, are not as clear. Y2R is expressed in primary afferent but not spinal cord neurons, with the greatest distribution on thinly myelinated afferent neurons (both Aδ- and C-nociceptors). The function of these Y2R-expressing dorsal root ganglion (DRG) neurons has been proposed to be pronociceptive, but this has not been rigorously tested. To begin to address this gap, we first asked whether intrathecal adminstration of the Y2R selective agonist, PYY_{3-36} (0.1, 1, 3 µg/5 µL) would reduce behavioral signs of persistent pain after plantar incision or spared nerve injury (SNI) in C57BL/6 male and female mice. Surprisingly, PYY_{3-36} had minimal effect on either mechanical or thermal hypersensitivity when tested 2 days after incision or 14 days after SNI (n = 6-8 per sex per surgery; P>0.05). One possible reason for this unexpected null result is that endogenous tonic NPY activation is high and the Y2Rs are at saturation; therefore, exogenous Y2 agonists have no available Y2Rs to bind to. To test if tonic basal Y2 activation was high, we administered the Y2R antagonist, BIIE0246 (0.01- 3.0 µg, 5 µL, i.t.) in naïve C57BL/6 male and female mice. BIIE0246 did not cause heat hypersensitivity or impairment of motor coordination but produced robust pain- and itch-like behaviors including mechanical and cold hypersensitivity, and biting (directed at the abdomen, back, and tail), rearing, face wiping, and scratching. To confirm these effects were mediated by Y2 receptors at the central terminals of primary afferent neurons, we bred a $Y2R^{DRG-/-}$ conditional knockout (cKO) by crossing $Pirt^{cre}$ mice (provided by X. Dong) with $Npy2r^{fl/fl}$ mice (provided by H. Herzog). As expected, the resulting $Y2R^{DRG-/-}$ cKO mice expressed Y2 mRNA in brain (hippocampus) but not DRG. BIIE0246-induced nociceptive and itch behaviors were observed in genetic controls, but not in $Y2R^{DRG-/-}$ mice. We suggest that endogenous NPY acts at Y2R on the central terminals of primary afferent neurons to tonically inhibit nociceptive and itch transmission under normal physiological conditions.

Disclosures: P. Basu: None. **A. Maddula:** None. **H. Herzog:** None. **T.S. Nelson:** None. **B.K. Taylor:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.05

Topic: D.03. Somatosensation – Pain

Support: R01DA37621
R01NS45954
R01NS62306

Title: Sex differences in protein kinase A signaling of the latent postoperative pain sensitization that is masked by kappa opioid receptors in spinal cord

Authors: *P. BASU[1], L. CUSTODIO-PATSEY[2], P. PRASOON[1], B. N. SMITH[2], B. K. TAYLOR[3];
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Abstract: Surgery leads to chronic postoperative pain (CPP) in 10-50% of patients (Kehlet et al., 2006). Although opioids can effectively treat acute postoperative pain, prolonged opioid therapy is controversial, and no other therapy provides sufficient analgesic efficacy for CPP. We used a very long-lasting model of CPP that involves latent sensitization (LS) of nociceptive neurons in the dorsal horn of the spinal cord and reported that LS is mediated by an N-methyl-D-aspartate receptor (NMDAR) > adenylyl cyclase-1 (AC1) > protein kinase A (PKA) and Exchange factor directly activated by cAMP (Epac) signaling pathway (Corder et al., Science 2013; Fu et al. 2019, 2020); however, these studies were performed in male mice only. To address this gap, we cut the plantar skin and underlying plantaris muscle in male and female mice, waited for behavioral signs of hyperalgesia to resolve, intrathecally injected the selective kappa opioid receptor-selective antagonist LY2456302 to unmask LS, and then evaluated behavioral and molecular signs of pain reinstatement. Interventions included AC1 knockout (KO) mice and pharmacological inhibitors or activators of NMDA (MK-801, 1 µg/5 µL, i.t.), AC1 (NB001, 1.5µg i.t.), PKA (H89, 10 nmol; 6-bnz, 10 nmol) and Epac1/2 (ESI-09, 10µg i.t.; 8-CPT, 3 nmol). LY2456302 but not saline vehicle reinstated mechanical hyperalgesia at the hindpaw even when administered 13 months after surgical incision, indicating that chronic pain vulnerability persists for over a year. When tested three weeks after incision in either male or female mice, we found that reinstatement of mechanical hyperalgesia was blocked with MK801, AC1 gene deletion, or NB001; the latter also attenuated LY2456302-evoked expression of the phosphorylated extracellular signal-regulated kinase (pERK), a marker of neuronal activation in the dorsal horn. Similarly, ESI-09 blocked hyperalgesia, while 8-CPT reinstated hyperalgesia in both sexes. Importantly, H89 prevented LY2456302-evoked reinstatement of mechanical hyperalgesia in male but not female mice, and 6-bnz reinstated postoperative hyperalgesia in male but not female mice. In neither sex did incision change the gene expression of KOR, GluN1, PKA, or Epac1 in the ipsilateral dorsal horn. We conclude that PKA-dependent mechanisms drive LS in a sex-dependent manner. Our results point to the development of AC1, PKA, and Epac inhibitors for the treatment of CPP, particularly PKA-selective inhibitors for men.

Disclosures: P. Basu: None. **L. Custodio-Patsey:** None. **P. Prasoon:** None. **B.N. Smith:** None. **B.K. Taylor:** None.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.06

Topic: D.03. Somatosensation – Pain

Title: In vivo effects of low intensity focused ultrasound in animal models of chronic pain

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Abstract: ABSTRACT: **Background**: Chronic pain affects 11% of the American population. Traditionally, neuromodulatory treatment for chronic pain is invasive and involves electrical stimulation. **Objective:** Here, we propose an alternative for lower extremity pain using low intensity external focused ultrasound (liFUS) administered to the L5 dorsal root ganglion (DRG).**Methods**: Male and female rats, as well as castrated male domestic swine were utilized to establish the efficacy of liFUS on altering sensory nociceptive thresholds (Von Frey filaments (VFF), hot plate test (HPT) and pain behavior (social/motor/affective scores) in a common peroneal nerve injury (CPNI) model of neuropathic pain. Documentation of sex differences in CPNI rats and of efficacy in a second neuropathic model- vincristine induced neuropathy (VIN)- in male rats further characterized liFUS effectiveness. Safety was assessed with locomotor testing and histological assessment. We gained initial insight into mechanism of action with electrophysiological studies and assessment of cytokine levels in the DRG and dorsal horn (DH) following liFUS. **Results**: LiFUS resulted in reduction of mechanical nociceptive thresholds (VFF) for 3 days in CPNI male rats ($p<0.001$). A similar reduction and time course was seen in VIN male rats ($p<0.001$). The duration of response was significantly longer in CPNI female rats whose VFF thresholds remained elevated for 7 days ($p<0.05$). HPT in both sexes of CPNI and VIN rats were increased for 24hr post treatment ($p<0.05$). Following liFUS, locomotion was intact in all rat groups and hematoxylin and eosin and fluoro-jade staining showed no evidence of damage from liFUS. After adaptation of the device to target
deeper structures and creation of a dose response curve, we were able to confirm our data in swine. VFF thresholds were increased for 5 days ($p<0.01$). Notably pain behavior, i.e. guarding, was reduced for up to 30 days following liFUS. Locomotion and histology again confirmed safety. LiFUS reduced common peroneal nerve latency for 35-55 minutes as compared to baseline in all groups ($p<0.05$). Cytokine measurements in male CPNI rats resulted in a decrease in pro-inflammatory cytokines in DRG 72h post-liFUS (TNFa, IL6, CNTF and IL1b, $p<0.001$). **Conclusion**: Taken together, we establish efficacy of liFUS to the L5 DRG in multiple species, models, and sexes. No functional or pathological damage occurred with the treatment. We hypothesize that liFUS results in alterations of the membrane of DRG cells resulting in immediate changes in CPN conduction and longer-term changes in cytokine levels which temporally correlate with allodynic changes.

Disclosures: A. Hellman: 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.; 5T32 GM075770. **P. Neubauer:** A. Employment/Salary (full or part-time):; Acoustic MedSystems. **G. Ghoshal:** A. Employment/Salary (full or part-time):; Acoustic MedSystems. **C. Burdette:** A.

Employment/Salary (full or part-time):; Acoustic MedSystems. **J.G. Pilitsis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Dr. Pilitsis is a consultant for Boston Scientific, Nevro, TerSera, and Abbott and receives grant support from Medtronic, Boston Scientific, Abbott, Nevro, TerSera, NIH 2R01CA166379-06 and NIH U44NS11.

Poster

P377. Treatments for Persistent Pain III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P377.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant T32 GM075770

Title: Contribution of small diameter non-peptidergic primary afferent neurons to central neuropathic pain in a new, more clinically relevant mouse model of multiple sclerosis

Authors: *K. L. NGUYEN[1], S. R. LAMERAND[2], R. P. DESHPANDE[3], B. K. TAYLOR[1]; [1]Dept. of Anesthesiol. and Perioperative Med., [2]Ctr. for Neurosciences, [3]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Over 50% of multiple sclerosis (MS) patients suffer from neuropathic pain (MSNP). Current treatments give inadequate relief due to incomplete understanding of underlying mechanisms. Recent electrophysiological recordings of primary afferent neurons (PAN) in the dorsal root ganglion (DRG) following experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, revealed increased afterhyperpolarization in small-diameter fibers. These data form the premise of our goal to understand the contribution of small-diameter (peptidergic or non-peptidergic) PANs to MSNP. Arguably the most common method to induce EAE is administration of myelin oligodendrocyte glycoprotein (MOG) to generate an autoimmune response targeting the myelin sheath. A MOG fragment is typically given with two adjuvants: complete Freund's adjuvant (CFA) to boost immunogenicity and pertussis toxin (PTX) to breakdown the blood-brain barrier and facilitate CNS immune cell infiltration. However, PTX can disrupt G-protein coupled receptors, cause pain, and alter autoimmune response gene expression. In 10-week-old C57BL/6 mice, we conducted the first rigorous comparison of a classic PTX EAE model with the novel non-PTX (nPTX) EAE model. We found that both PTX and nPTX EAE mouse models showed the same degree of: 1) motor deficits; 2) plantar hindpaw mechanical and cold hypersensitivity (except cold hypersensitivity resolved more quickly after PTX EAE than nPTX EAE); and 3) lumbar spinal cord demyelination. Unlike most rodent models of MS including PTX EAE, the nPTX EAE group exhibited somatosensory cortex demyelination, a core feature of MS in human patients and cold hypersensitivity. We suggest

nPTX EAE to be the most clinically relevant rodent model available to study not only MSNP, but MS in general. To evaluate the contribution of peptidergic and non-peptidergic neurons to MSNP, we induced nPTX EAE. After 12 days we administered capsaicin (10µg/mouse, i.t.) or IB4-saporin (1.5µg/mouse, i.t.) to primarily ablate peptidergic or nonpeptidergic C-fibers, respectively. Ablation efficacy was successfully confirmed with dramatic loss in DRG of TRPV1/CGRP immunoreactivity (peptidergic C-fibers) following capsaicin, and IB4 immunoreactivity (nonpeptidergic C-fibers) following IB4-saporin. IB4-saporin, but not capsaicin, partially reduced mechanical hypersensitivity and reversed cold hypersensitivity within 9 days. These data suggest nonpeptidergic but not peptidergic C-fibers contribute to MSNP. Our next studies will use genetic knockout, chemogenetic, and optogenetic strategies using $Mrgprd^{CreER}$ mice to modulate the activity of nonpeptidergic C-fibers.

Disclosures: K.L. Nguyen: None. **S.R. Lamerand:** None. **R.P. Deshpande:** None. **B.K. Taylor:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.01

Topic: D.07. Vision

Support: H2020-MSCA-IF-2016 749697 GaMePLAY
PRIN2017 2017HMH8FA

Title: The gut microbiota of environmentally enriched mice regulates visual cortical plasticity

Authors: *S. CORNUTI[1], L. LUPORI[1], R. MAZZIOTTI[2], E. BORGHI[3], E. OTTAVIANO[3], G. SAGONA[4], T. PIZZORUSSO[1], P. TOGNINI[5];
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Abstract: Exposing animals to an enriched environment (EE) has dramatic effects on function and plasticity of many different brain areas, including sensory systems. For example, ocular dominance (OD) plasticity, a change in the eye preference of cortical cell responses induced by monocular deprivation (MD), usually present only during a critical period of postnatal development, can be observed in adult mice raised in EE. Classically, the beneficial effects of EE on brain physiology have been explained through mechanisms that arise from the central nervous system (CNS) itself: increased hippocampal neurogenesis, changes in the excitatory/inhibitory circuit balance, structural plasticity of dendritic spines, and others. In our work, the focus has

been shifted to signals coming from the periphery of the body, providing a novel and unexpected link between the gut microbiota and EE-driven plasticity. Indeed, the intestinal microbiota has emerged as a regulator of system-wide physiology, such as immune system development, maintenance of metabolic homeostasis, and moreover, recent evidence shows that the influence of gut microbes goes beyond the periphery towards the CNS. However, little is known about how intestinal bacteria impinge on neuronal function and plasticity. In particular, the existence of a link between experience-dependent plasticity and the gut microbiota has yet to be explored. In our study, we found that EE shaped the intestinal bacteria composition. A longitudinal analysis (P20, P25, P90) of the fecal microbiota composition through 16S rRNA sequencing revealed striking differences between EE and standard (ST) raised animals, especially during adulthood. The depletion of the gut microbiota using an antibiotic cocktail (ABX) was able to prevent OD plasticity and to influence microglia morphology. Using longitudinal 2-photon imaging, we observed also a change in spine dynamics and density in EE+ABX mice compared to EE condition. Furthermore, a mass spectrometry analysis of fecal short-chain fatty acids (SCFAs) - metabolic byproducts of the gut microbiota known to influence brain function - revealed a significant increase in acetate, butyrate and propionate in EE mice. SCFA treatment alone promoted adult OD plasticity and influenced microglia morphology in ST mice suggesting that SCFA could act as a gut-brain signal to promote EE-driven plasticity. Strikingly, transferring the microbiota of EE donor mice to ST mice through fecal microbiota transplant was able to activate adult OD plasticity in the ST recipients. Overall, our work shows that experience-dependent changes in gut microbial composition are able to regulate brain plasticity.

Disclosures: S. Cornuti: None. **L. Lupori:** None. **R. Mazziotti:** None. **E. Borghi:** None. **E. Ottaviano:** None. **G. Sagona:** None. **T. Pizzorusso:** None. **P. Tognini:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.02

Topic: D.07. Vision

Support: James S. McDonnell Foundation 220020516-0

Title: Effects of early blindness on cortical and thalamic connections of multi-modal cortex in the short-tailed opossum (Monodelphis domestica)

Authors: *C. PINEDA[1], L. A. KRUBITZER[2];
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Abstract: Early changes in sensory input produce dramatic effects on the development of the neocortex. In congenitally blind humans, correlative evidence demonstrates cross-modal

plasticity occurs in the occipital cortex, which is active under several conditions, including during reading braille, auditory-spatial processing, and language processing. Direct evidence from animal models paints a more detailed picture of the changes occurring in the occipital cortex. Studies from our laboratory examining the early loss of vision take advantage of the highly altricial lifestyle of the short-tailed opossum (*Monodelphis domestica*). By bilaterally enucleating opossums at post-natal day 4 (P4), before the formation of retinogeniculate and thalamocortical pathways, we have demonstrated changes in the function and connectivity of the occipital cortex and other spared primary sensory areas, such as the primary somatosensory cortex. These findings recapitulate the cross-modal plasticity in the occipital cortex of early blind humans and illustrate that the loss of input from one primary sensory system has broad effects on the spared sensory systems at both cortical and subcortical levels. No one has examined the consequences of the early loss of vision on higher-order associative areas of the cortex; an area important for multi-sensory integration. We compared the connectivity of higher-order areas of the cortex of sighted and early blind opossums bilaterally enucleated at P4 using neuroanatomical tracing and histological techniques. First, we generated a standard set of coordinates relative to the opossum bregma to target our tracer injections. Then we examined the patterns of organization of cortico-cortical and thalamocortical connections of higher-order cortical area in the sighted short-tailed opossum. Characterizing the normal organization of higher-order cortical areas allowed us to compare the pattern of cortico-cortical and thalamocortical of blind and sighted short-tailed opossums. These data contribute to the growing body of knowledge about the possible cortical modifications in congenitally blind individuals.

Disclosures: C. Pineda: None. **L.A. Krubitzer:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.03

Topic: D.07. Vision

Title: Neural mechanisms of short term monocular deprivation in primary visual cortex

Authors: R. A. MILLER, III, *D. Y. TS'O;
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Abstract: Maintaining the appropriate interocular balance, the weighting of left and right eye neural signals to the central visual pathways, is an important prerequisite for achieving the advantages of binocular vision. An altered interocular balance has been demonstrated experimentally in humans and animals following a short-term (1-3 hours) monocular deprivation (STMD). The surprising outcome is a non-Hebbian relative increase in the deprived eye (DE) gain post-deprivation, which in previous psychophysical studies may last up to 90 minutes. The

neural mechanisms underlying this STMD effect are largely unknown. We set out to reveal possible neural mechanisms underlying STMD in the responses of primate V1 neurons. Before, during and after a 1-2 hour STMD of one of the two eyes in adult macaque monkeys, single-unit V1 responses were recorded using a 32-channel linear multi-electrode array. Following the STMD, in a sample cell population (N=155), the single-unit responses revealed an average relative gain of the deprived eye (DE) responses over the non-deprived eye (NDE) responses of 29% as compared with their respective pre-STMD levels. The DE responses remained elevated for about 25 minutes, after which they returned to their pre-STMD levels. In contrast, the post-STMD NDE responses were depressed as compared with their pre-STMD levels, even beyond 25 minutes, thus suggesting possible differences in the time courses of the DE vs NDE STMD effects. Although the supragranular cell population exhibited more robust STMD effects, cells impacted by STMD were found in all V1 cortical layers and constituted 46% of the sample. Preliminary results indicated that extending the duration of the STMD period to 7.5 hours significantly enhanced the STMD effect. Overall, these results from single-unit recordings in primate V1 under the STMD protocol match several key features of the strength of STMD effect reported in the psychophysical literature, and indicate an important contribution to the STMD effect within the V1 circuitry.

Disclosures: R.A. Miller: None. **D.Y. Ts'o:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.04

Topic: D.07. Vision

Support: R21-EY031265 to H-KL & GMH
Kavli NDI Postdoctoral Fellowship to LM
Johns Hopkins University Discovery Award to H-KL & GMH

Title: Non-invasive Low-Intensity Focused Ultrasound (LIFU) stimulation to promote visual cortex plasticity

Authors: *L. MESIK[1,2,4], M. CONGEDO[5], B. D. GRIER[1,2], D. SEVERIN[2,1], G. EWALL[1,2], S. PARKINS[3], T. HARRIGAN[5], K. SCHULTZ[5], G. M. HWANG[5,4], H.-K. LEE[1,2,4,3];
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Abstract: Transcranial ultrasound stimulation has emerged as a promising non-invasive brain stimulation method, which has in principle several advantages compared to other more established non-invasive techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). TMS and tDCS have rather poor spatial resolution (in the range of ≥ 1 cm) and are limited to cortical activation. Deep brain stimulation is currently done via invasive electrode implantations. In contrast, transcranial ultrasound stimulation has a more refined spatial resolution (in the mm range) and has the potential to activate deep brain structures. Several studies have reported that transcranial ultrasound stimulation can induce muscle activity, sensory responses, or behavioral changes. In addition, it has been demonstrated that ultrasound activates neurons by eliciting action potentials. However, the biological consequences of ultrasound stimulation are less understood.
Here we demonstrate that low-intensity focused ultrasound (LIFU) stimulation targeted to a deep brain structure, the visual thalamus (dLGN), leads to long-term synaptic plasticity of the thalamocortical inputs to the primary visual cortex (V1) of adult mice. To demonstrate this, we expressed channelrhodopsin2 (ChR2) bilaterally in dLGN and stimulated the dLGN of one hemisphere via transcranial LIFU stimulation (0.54 MHz transducer, 50 c/p, 0.5 kHz PRF) for 1 hour under anesthesia. Mice were bilaterally enucleated prior to the LIFU stimulation to prevent visually-driven activity. Synaptic transmission from ChR2 expressing dLGN axons to V1 layer 4 (L4) neurons were measured *ex vivo* in acute V1 slices several weeks after the initial activation. We found significant depression of thalamocortical synaptic transmission in the stimulated hemisphere compared to the non-stimulated control hemisphere. Our results suggest that non-invasive LIFU stimulation can produce long-term plasticity of thalamocortical synapses in the adult V1. We are currently testing different parameters of LIFU stimulation to determine whether we can drive bidirectional long-term plasticity.

Disclosures: L. Mesik: None. **M. Congedo:** None. **B.D. Grier:** None. **D. Severin:** None. **G. Ewall:** None. **S. Parkins:** None. **T. Harrigan:** None. **K. Schultz:** None. **G.M. Hwang:** Other; This material is based upon work of GMH supported by (while serving at) the National Science Foundation. Any opinion, findings, and conclusions or recommendations expressed in this material are those, of the author(s) and do not necessarily reflect the views of the National Science Foundation.. **H. Lee:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.05

Topic: D.07. Vision

Support: KU Leuven C14/17/109

FWO Flanders G0D5817N, G0B8617N, G090714N, G0E0520N, G088418N and Odysseus G0007.12
Horizons 2020 945539

Title: Pairing a visual stimulus with micro-stimulation of the ventral tegmental area increases neural responses on the short term in higher-order visual cortex

Authors: *J. HERPERS[1], W. VANDUFFEL[2], R. VOGELS[1];
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Abstract: Electrical microstimulation of the dopaminergic ventral tegmental area (VTA-EM) paired with a task-irrelevant visual stimulus feature increases its representation in the posterior inferior temporal cortex (PIT) at fMRI (Arsenault & Vanduffel, Nature Communications, 2019) and spiking level (Herpers et al., VSS, 2021). These studies suggest that VTA-EM-stimulus pairing is sufficient to induce cortical plasticity in visual cortex. However, the latter studies have only employed longer periods (multiple days) of VTA-EM-stimulus pairing. Here, we investigated whether VTA-EM-stimulus pairing also induces short term plasticity using VTA-EM and electrophysiological recordings in PIT. 2 monkeys performed passive fixation tasks while alternating between multi-unit activity (MUA) recordings or VTA-EM-stimulus pairing epochs. 4 fractal color images were used in each daily session. The PIT recordings alternated with multiple 20-minute VTA-EM epochs (~60 VTA-EM events), where one fractal image was paired with VTA-EM (STIM) while another fractal was presented as control (CON). Two other fractals (dummies) were presented only during the recordings. In both monkeys, we observed a general response reduction to all 4 fractals over the 6-7 recording epochs within a day (with alternating VTA-EM epochs), likely reflecting repetition suppression. After the first VTA-EM epoch, the CON fractal that was presented during the VTA-EM epoch, but not paired with VTA-EM, showed the largest decrease in response. When comparing the relative change in response (STIM-CON) between the recording epochs before the first VTA-EM pairing and the first post-VTA-EM recording epoch, we found a significant increase in relative firing rate to the paired fractal. This effect persisted thoughout the day but did not increase after subsequent VTA-EM epochs. The relative increase in spike rate for STIM-CON might reflect reduced repetition suppression induced by increased responses to the STIM fractal after VTA-EM-pairing. In one monkey only, the relative Fano factor and noise correlations were lower for the STIM-CON pair post-VTA-EM compared to the dummy pair.In conclusion, pairing a fractal image with VTA-EM during passive fixation can influence short term neural response properties in area PIT.

Disclosures: J. Herpers: None. **W. Vanduffel:** None. **R. Vogels:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.06

Topic: D.07. Vision

Support: F31EY028431

Title: Bidirectional regulation of pyramidal neuron excitability is required for reactivation of ocular dominance plasticity following dark exposure and light reintroduction

Authors: *A. BORRELL, E. M. QUINLAN;
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Abstract: Amblyopia is a common deficit in visual acuity induced by monocular visual impairment during a “critical period” (CP) of early development when plasticity in visual cortical (V1) circuits is high. Following the CP, both the induction and reversal of amblyopia is difficult, due in large part to the reduction in synaptic plasticity with age. We have shown previously that visual deprivation via dark exposure (DE) followed by light reintroduction (LRx) robustly reactivates juvenile-like plasticity in mouse V1. DE is thought to increase the firing rates of pyramidal neurons in V1 by increasing the expression of GluN2b containing NMDARs (He et al., 2006) and lowering the threshold for Hebbian plasticity at cortico-cortical synapses (Bridi et al., 2018). In addition, DE lowers the threshold for activation of MMP-9-dependent perisynaptic proteolysis at thalamo-cortical synapses, and rapid engagement by LRx is predicted to decrease excitatory drive to the cortex (Murase et al., 2019). Here we test the hypothesis that a decrease in V1 pyramidal neuron excitability induced by LRx is necessary for the reactivation of plasticity following DE in adults. We used targeted, viral-mediated expression of inhibitory chemogenetic Gi-DREADD receptors to maintain hyper-excitability of pyramidal neurons through disinhibition during DE/LRx in adult (P100+) PV-cre mice. Chronically implanted electrode arrays allowed simultaneous recording of single unit (SU) spiking output and visual-evoked local field potentials (VEPs) in V1 of awake head-fixed mice. We found that chemogenetic disinhibition of V1 significantly increased VEP amplitude (19% ±12) and regular spiking (presumptive pyramidal cell) SU spike rates (41% ± 20) across all layers (mean ± SEM) in Gi-DREADD infected, but not uninfected PV-cre control mice. Brief (3 day) monocular deprivation (MD) following LRx induced a significant negative shifts in the ocular preference of cortical responses, indicative of the reactivation of ocular dominance plasticity, in PV-cre control mice (ks test, $p = 3.68e\text{-}4$), but not Gi-DREADD infected mice. In contrast, chemogenetic disinhibition of V1 did not prevent the MD-induced reduction in acuity in response to brief MD (-28% ± 16) consistent with different anatomical loci for these two forms of experience-dependent plasticity (Stephany et al., 2014). This suggests that bidirectional regulation of pyramidal neuron excitability is required for the reactivation of ocular dominance plasticity in adults by DE/LRx.

Disclosures: A. Borrell: None. **E.M. Quinlan:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.07

Topic: D.07. Vision

Support: NIH Grant EY016431
NIH Grant EY025922
NIH Grant DC009607

Title: Chronic monocular deprivation reveals MMP9-independent and -dependent aspects of murine visual system maturation and plasticity

Authors: *S. MURASE[1], S. E. ROBERTSON[2], C. L. LANTZ[1], J. LIU[1], D. E. WINKOWSKI[1], P. O. KANOLD[1], E. M. QUINLAN[1];
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Abstract: The maturation of the mouse visual system includes experience-independent emergence of stimulus selectivity followed by experience-dependent refinement of receptive fields and acuity. Here we combine deletion of the matrix metalloproteinase MMP9 with chronic monocular deprivation (cMD) to ask which aspects of visual system maturation require the activity of this proteinase. Calcium imaging of supragranular neurons of the binocular region of primary visual cortex (V1b) of adult (>P90) wild type (WT) mice of both sexes, following delivery of AAV1.GCaMP6s to V1b (AP: 1.0 mm, MD: -3.0 mm, DV: 0.3 mm, right hemisphere for binocular C57BL/6J and MMP9$^{-/-}$ mice or hemisphere contralateral to cMD eye) demonstrated that cMD initiated at eye opening (P14) resulted in a significant reduction in the contralateral bias (CBI, (C-I)/(C+I), 0.73±0.05 for normal-reared (NR), 0.40±0.03 for cMD, *p=6.8 x 10-4, Student's T-test, n = 5 subjects), and a decrease in the strength of deprived eye visual responses to all stimulus contrasts and spatial frequencies. Despite chronic MD, V1 neurons retained selectivity for orientation ((1-CV), 0.25±0.03 for NR, 0.29±0.04 for cMD, *p<0.05, Student's T-test, n = 59, 51 neurons), direction ((1-dirCV), 0.21±0.03 for NR, 0.38±0.04 for cMD, *p<0.05, Student's T-test, n = 59, 51 neurons) and spatial frequency (mean peak SF, 0.17±0.01 cpd for NR, 0.16±0.02 cpd for cMD, p=0.47, Student's T-test, n = 5 subjects), but direction selectivity was lower in neurons tuned to high spatial frequency (>0.05 cpd) visual stimuli ((1-dirCV), 0.27±0.02 for NR, 0.20±0.02 for cMD, *p<0.05, Student's T-test, n = 94, 95 neurons). Constitutive deletion of MMP9 did not appear to impact the development of stimulus selectivity, as adult MMP9$^{-/-}$ mice had normal spatial frequency (mean peak SF, 0.16±0.04 cpd for NR, 0.11±0.003 cpd for cMD, p=0.47, Student's T-test, n = 5 subjects), orientation and direction selectivity ((1-CV), 0.25±0.02 for NR, 0.24±0.02 for cMD, p=0.44; (1-dirCV), 0.20±0.01 for NR, 0.19±0.01for cMD, p=0.50, Student's T-test, n = 104, 160 neurons). However, MMP9$^{-/-}$ mice were insensitive to plasticity engaged by cMD (CBI, 0.66±0.06 for NR, 0.70±0.02 for cMD, p=0.56, Student's T-test, n = 6 subjects), but retained other forms of experience-dependent synaptic plasticity such as stimulus-selective response potentiation (SRP, familiar: 120.4±4.7% of initial, *p=0.03, novel: 108.7±5.8% of initial, p=0.16, Student's T-test,

n = 5 subjects). Our findings demonstrate that MMP9 is obligatory for reorganization of V1b induced by cMD, but dispensable for other forms of experience-independent and -dependent synaptic plasticity.

Disclosures: S. Murase: None. **S.E. Robertson:** None. **C.L. Lantz:** None. **J. Liu:** None. **D.E. Winkowski:** None. **P.O. Kanold:** None. **E.M. Quinlan:** None.

Poster

P476. Plasticity Mechanisms in the Visual System

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P476.08

Topic: D.07. Vision

Support: Office of Naval Research ILIR award

Title: Effects of Acute Hypoxia Exposure on Early Visual and Auditory Processing as Measured with EEG

Authors: *K. J. BLACKER[1], D. G. MCHAIL[2];
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Abstract: Hypoxia continues to be a significant aeromedical risk factor. Recent work has demonstrated that acute hypoxia disrupts automatic visual and auditory processing that can be detected at a neural level using EEG. Specifically, passively evoked ERPs were found to track the impairment of sensory processing during a hypoxic exposure. Further exploration of ERPs that occur earlier in the sensory processing stream may provide a more robust indicator of impairment and be developed as an early detection system for hypoxic threats to aircrew. Here, two early sensory ERPs were examined in response to hypoxia: visual and auditory ERPs. Participants underwent three separate experimental sessions: 1) a normoxia baseline where participants were presented with visual and auditory stimuli, separately, 2) a hypoxia session with visual stimuli presented, and 3) a hypoxia session with auditory stimuli presented. Normobaric hypoxia exposures (9.7% O_2) were delivered via a reduced oxygen breathing environment. For the visual paradigm, participants were shown a reversing black and white checkerboard stimulus and the P100 component was measured as the response to the pattern reversal every 1 second. The P100 is thought to reflect the integrity of visual information processing and arises from neural activity in primary visual cortex. For the auditory paradigm, participants were presented with pairs of auditory tones and a series of waveforms that index different stages of sensory gating were measured including the P50, N100, and P200. Typically, there is a reduction in the amplitude of these components in response to the second tone in the pair, which is known as sensory gating and reflects efficient filtering of redundant information. Under hypoxic conditions, compared to normoxia, there was a significant reduction in the

amplitude of the visual P100 component. This difference represented a 25% reduction in amplitude. For the auditory paradigm, there was no difference in sensory gating for the P50 or N100 between hypoxia and normoxia; however, the P200 component showed a trend toward lower gating scores under hypoxic conditions. Our results here are consistent with previous work showing that passively elicited ERPs in response to both visual and auditory stimuli are disrupted during hypoxia. Notably, we found a robust effect in the visual domain suggesting that the P100 may be a viable candidate as a neural biomarker of visual impairment during hypoxia. In the auditory domain, only the P200 component showed changes with hypoxia, which is consistent with previous work showing that auditory processing is affected at later stages of processing compared to the visual system.

Disclosures: K.J. Blacker: None. **D.G. McHail:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.01

Topic: E.04. Voluntary Movements

Support: VA ORD Award I01RX002619-01A2

Title: The behavioral relevance of aging-related changes to motor cortical receptor dynamics

Authors: *T. S. NOVAK[1], J. R. NOCERA[1,2], L. C. KRISHNAMURTHY[1,3], K. M. MCGREGOR[1,2];
[1]Ctr. for Visual and Neurocognitive Rehabil., Atlanta Veterans Admin. Med. Ctr., Decatur, GA; [2]Neurol., Emory Univ., Atlanta, GA; [3]Physics & Astronomy, Georgia State Univ., Atlanta, GA

Abstract: The inhibitory receptor dynamics of cortico-motor circuitry demonstrate remarkable change as a function of normal aging. However, to date, there is a lack of consensus on the functional significance of these dynamics in the context of adaptive motor skill acquisition. In the current study, we used paired-pulse transcranial magnetic stimulation (TMS) to examine intra-cortical inhibitory function in a sample of 17 healthy older adults (mean=65 ±4 years). Short intracortical inhibition (SICI) and long intracortical inhibition (LICI) TMS measures were evaluated at rest and during submaximal voluntary contraction to examine individual aging differences in state-dependent intracortical inhibition. Subjects were then split into subgroups where they performed a single session of motor sequence learning under a unique set of sensorimotor demands (low demand: 8 button sequence at 1000ms intervals (n=9); high demand: 12 button sequence at 500ms intervals (n=8)). SICI values varied significantly between subjects in both resting and event-related TMS conditions, with the sample distribution primarily exhibiting cortical facilitation. LICI values were less variable, however, the group distribution

indicated a presence of subject-dependent inhibition or facilitation with long-interval paired pulse-stimulation. Interestingly, SICI and LICI values were inversely correlated with performance adaptations (accuracy and reaction time) in the early stage of motor practice for both groups, meaning subjects with higher values of event-related facilitation performed sequential learning faster and more accurately. Moreover, correlations were consistently stronger in the high demand practice condition. The conclusions are three-fold: Relations between cortico-motor receptor function and behavioral outcomes may be best established in the context of an event-related cortical state; sufficient task constraints should be considered to address individual differences in the aging motor system; and increases in state-dependent excitability levels might act as a compensatory mechanism for older adults to quickly acquire novel motor skills. Provided motor skill acquisition is a complex, non-linear process, aging-related cortical mechanisms underlying unique timescales of sensorimotor adaptation requires further investigation.

Disclosures: T.S. Novak: None. **J.R. Nocera:** None. **L.C. Krishnamurthy:** None. **K.M. McGregor:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.02

Topic: E.04. Voluntary Movements

Support: National Research Foundation of Korea Grant 2017S1A20322920

Title: The effects of a cognitive walking program on improving motor function of the brain in older adults

Authors: *J. WANG[1], S.-J. KANG[2];
[1]Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [2]Sangmyung Univ., Seoul, Korea, Republic of

Abstract: Aging is typically associated with a decline in both cognitive and motor functions of the brain. However, slowing or reversing that process may be possible through physical activity. We recently demonstrated that a walking-based indoor exercise program, called a ‘cognitive walking program’ (CWP), has beneficial effects for improving cognitive function in both physically active and sedentary older adults (Kang et al. 2021). However, it is unknown whether the same exercise program is also beneficial for improving motor function of the brain. In the current study, we investigated the effects of CWP on motor function of the brain by examining changes in the pattern of interlimb transfer following visuomotor adaptation in physically active and sedentary older adults. Healthy older adults were divided into two groups based on their

physical activity level: active and sedentary. All subjects in both groups participated in the CWP for 6 months (60 min/session, 3 sessions/week). To assess motor function of the brain, we employed a visuomotor adaptation task and examined the pattern of interlimb transfer, in terms of initial direction errors, following visuomotor adaptation. It was hypothesized that if the CWP had beneficial effects, the pattern of transfer would change from a symmetrical (i.e., transfer from the left to the right arm, and vice versa) to an asymmetrical pattern (i.e., transfer only from the left to the right arm) with the progress of the CWP, as suggested in our previous studies (Wang et al. 2011, 2016). Results showed that transfer of visuomotor adaptation occurred symmetrically in both subject groups before the subjects began the CWP, and that the symmetrical pattern was maintained even after 6 months. There was no significant difference between the two groups. These results suggest that the CWP may have beneficial effects for improving cognitive function of the brain in older adults, but not for improving motor function of the brain in older adults, at least when the motor function is reflected as the changes in the pattern of interlimb transfer following visuomotor adaptation.

Disclosures: J. Wang: None. **S. Kang:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.03

Topic: E.04. Voluntary Movements

Title: Facilitative effects of use-dependent learning on interlimb transfer of visuomotor adaptation in a person with congenital mirror movements

Authors: *Y. JO, M. JAVIDIALSAADI, J. WANG;
Dept Kinesiology, Univ. of Wisconsin, Milwaukee, WI

Abstract: It has been shown that use-dependent learning can facilitate interlimb transfer of motor learning in neurologically intact individuals. However, it is unknown whether it can also facilitate interlimb transfer in individuals with neurological impairment. Here, we examined the effect of use-dependent learning on interlimb transfer of visuomotor adaptation in a person with congenital mirror movements, DB, who showed no interlimb transfer previously (Bao et al. 2020; Javidialsaadi and Wang 2021). In the current study, DB first performed reaching movements with the right arm repeatedly while adapting to a visuomotor 30-degree counterclockwise rotation condition with the left arm, and then adapted to the same rotation condition with the right arm, which resulted in substantial transfer. The results show that the percentage of interlimb transfer was over 90%, which is similar to that observed in healthy young adults in our previous study results. These findings suggest that visuomotor adaptation involves two learning mechanisms: model-based learning, which is effector independent; and

use-dependent (or model-free) learning, which is effector specific. These findings also suggest that whereas both types of mechanisms can contribute to interlimb transfer of motor learning, the relative contribution of use-dependent learning can be at least as large as that of model-based learning (if not greater). Collectively, these findings indicate potential benefits of use-dependent learning for neurorehabilitation in individuals with brain lesions (e.g., cerebellar damage).

Disclosures: Y. Jo: None. **M. Javidialsaadi:** None. **J. Wang:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.04

Topic: E.04. Voluntary Movements

Support: NIH NINDS NS116883
Foundation for Physical Therapy Research

Title: Parkinson's disease does not impair implicit sensorimotor adaptation

Authors: *J. TSAY, T. NAJAFI, L. SCHUCK, T. WANG, R. IVRY;
Psychology, UC Berkeley, Berkeley, CA

Abstract: Whether implicit motor adaptation is impacted by Parkinson’s Disease (PD) remains a topic of debate, an issue that is of particular relevance given increasing evidence of interactions between reward- and error-based learning processes. Several early studies reported impaired implicit adaptation in PD, whereas recent work failed to observe deficits in this group. There is a range of methodological differences making it difficult to reconcile these disparate results. Moreover, most studies have used conventional sensorimotor adaptation tasks that fail to isolate implicit adaptation from the influence of explicit learning processes. We revisited this problem using a clamped feedback method that isolates implicit adaptation. In Experiment 1, participants reached to one visual target, while on each trial the angular position of the feedback cursor was shifted from the target (not the hand) by either 3°, 10°, 30°, or 45°, with the direction (CW or CCW) randomized. Implicit adaptation was indexed by the change in hand angle from trial n to trial n + 1. By sampling a wide range of visual errors, we could assess whether PD and their age-matched controls differ in their error sensitivity. For both groups, the adapted response scaled with the error size between 3° and 30°, and then saturated. Importantly, the function was similar for the PD and controls. In Experiment 2, participants reached to one of two locations on each trial. For each location, the direction (CW vs CCW, varied between subjects) and the size (3° or 30°, varied by location within-subjects) was fixed for 150 trials. In this manner, the effects of implicit adaptation to each invariant perturbation will accumulate over trials; as such, any subtle group differences should be magnified. The rate of adaptation was faster in response to the larger

clamp and reached a higher asymptotic value (collapsed over groups: 3° = 13.7°; 30° = 26.4°). Again, the PD and controls did not differ in terms of rate or asymptote. These null results motivated us to perform a meta-analysis of implicit adaptation and PD. We restricted the analysis to studies that reported an aftereffect, a measure that best reflects the degree of implicit adaptation without contamination from other learning processes. The effect size ranged from -2.28 to 1.09 (negative = attenuated adaptation relative to controls, positive = enhanced), with significant heterogeneity between studies (I^2 = 62%, $p < 0.01$). The mean effect size of -0.36 (95% CI = [0.03, -0.75]) was not significant, with 10 of the 15 studies including 0, the null hypothesis. Taken together, the results from the current experiments and the meta-analysis indicate that PD does not impact implicit adaptation.

Disclosures: J. Tsay: None. **T. Najafi:** None. **L. Schuck:** None. **T. Wang:** None. **R. Ivry:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.05

Topic: E.04. Voluntary Movements

Support: CRSNG

Title: Post-learning alcohol ingestion as a pharmacological agent to investigate the mechanisms of motor memory consolidation

Authors: *O. DEMERS[1], R. HAMEL[2], J.-F. LEPAGE[3], P.-M. BERNIER[4];
[1]Univ. de Sherbrooke, Hâvre aux Maisons, QC, Canada; [2]Physiologie, Univ. de Sherbrooke, Sherbrooke, QC, Canada; [3]Pediatrics, Sherbrooke Univ., Sherbrooke, QC, Canada; [4]Dept. de Kinanthropologie, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Memory consolidation refers to the entirety of neuroplastic phenomena that occur following learning allowing for newly formed memories to transform into long-term memories. However, the mechanisms of this process for human motor memories remain unknown. Interestingly, human studies on declarative memories indicate that pharmacological interventions seeking to increase GABAergic activity immediately post-learning dose-dependently enhance memory consolidation. This study sought to evaluate if such intervention can also enhance motor memory consolidation by testing the following hypothesis: post-learning alcohol consumption - a pharmacological agent known to increase GABAergic activity, amongst other actions - will dose-dependently enhance motor memory consolidation as compared to a placebo condition. To that purpose, a fully within-subject and counterbalanced design was carried out where participants (n = 24; 12 males) had to adapt to a gradually introduced visual deviation and then

ingest a beverage that either contained no (Placebo), a Moderate or High alcohol dose. The alcohol doses were sex- and body weight-adjusted to reach a peak blood alcohol concentration of 0.000%, ~0.050%, and ~0.100% for the Placebo, Moderate, and High conditions, respectively. To assess consolidation, retention was assessed 24h later through reach aftereffects. Preliminary results (n = 22) revealed that, contrary to the hypothesis, alcohol did not influence consolidation. Specifically, long-term retention in both the Moderate and High conditions - on average - did not meaningfully differ from the Placebo condition (all Cohen's $d_z < 0.150$).

Disclosures: O. Demers: None. **R. Hamel:** None. **J. Lepage:** None. **P. Bernier:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.06

Topic: E.04. Voluntary Movements

Title: Age-related Changes in Electrophysiological Correlates of Visuomotor Adaptation

Authors: *A. T. WATRAL[1], I. FLINT[1], L. RAJESHKUMAR[2], M. SAGUE[1], K. TREWARTHA[1];
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Abstract: Motor learning is known to progress on multiple timescales with initial rapid improvements inperformance, followed by more gradual improvements through repeated practice. Thesetimescales have been attributed to two processes that learn and forget at different rates: A fastprocess allows performance improvements to occur rapidly by adapting strongly to errors, and aslow process that responds to errors more gradually and provides slower gains in performanceover time. This latter process may contribute to solidification of a motor memory. This impliesthat different neurocognitive mechanisms are responsible for initial motor learning driven by thefast process and more gradual learning driven by the slow process. While the neuralmechanisms associated with motor learning are not fully understood, previous researchsuggests that a neurophysiological signature of context updating and learning - the P300 event-related potential (ERP) component - diminishes across learning in younger adults. Aging isassociated with diminished visuomotor adaptation, particularly in the earliest phase of learning.To examine the neurophysiological correlates of age-related changes in visuomotor adaptation,we compared the modulation of the P300 across learning between healthy younger and olderadults. Continuous electroencephalogram (EEG) recordings were acquired while participantsperformed a visuomotor rotation (VMR) task. Younger adults adapted to the rotation better thanolder adults, particularly in the earliest phase of adaptation, consistent with previous research.ERP analyses revealed that a posterior P300 (P3b) component was elicited by the

targetsduring the VMR task and the magnitude of this component was reduced across adaptation inthe younger participants. This modulation was less pronounced in the older participants. TheP3b was also larger in the first quarter of the adaptation trials in younger compared to olderadults suggesting that the ability to update working memory with task context informationfacilitated better early learning in younger compared to older adults. These observations provideinsight into the neural basis of age-related changes in sensorimotor adaptation. Modulation ofthe magnitude of the P3b across learning may reflect a shift from fast process-mediatedlearning to slow-process mediated learning and suggests that the neurocognitive mechanismssupporting performance change as adaptation unfolds.

Disclosures: A.T. Watral: None. **I. Flint:** None. **L. Rajeshkumar:** None. **M. Sague:** None. **K. Trewartha:** None.

Poster

P531. Reaching Control: Motor Learning: Human Neurophysiology and Pathology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P531.07

Topic: E.04. Voluntary Movements

Support: VA ORD Award I01RX002619-01A2

Title: Changes GABA concentration in the sensorimotor cortex in older adults during motor skill acquisition

Authors: *G. CHAMPION[1], J. R. NOCERA[2], L. C. KRISHNAMURTHY[2], T. S. NOVAK[2], K. MAMMINO[1], K. MCGREGOR[1];
[1]Georgia State Univ., Atlanta, GA; [2]DVA, Decatur, GA

Abstract: Aging is associated with changes in cortical excitability which may affect motor learning *via* selective modulation of gamma aminobutyric acid (GABA). Previous studies using magnetic resonance spectroscopy (MRS) to measure GABA in younger adults found that GABA in the sensorimotor cortex (M1S1) tended to decrease during the execution of a repeated motor sequence. We are not aware of any studies investigating changes in dynamic GABA in older adults, who evidence slower and more variable performance during motor skill acquisition. The dynamic change in GABA density in M1S1 in older adults is currently unknown and represents a critical gap in our understanding of behavioral performance to neurometabolite changes during motor learning. As such, the purpose of the current study is to quantify changes in cortical GABA during motor learning in the aging brain. We hypothesize that older adults with greater dynamic range in M1S1 GABA levels will display more efficient motor learning. We report on a total of 14 healthy older adults aged 62 to 80 years (M = 69.71, SD = 5.68, nine females). Using MRS at 3T, we measured changes in GABA concentration in M1S1 during an eight or 12 finger-

movement motor entrainment (2 or 3 runs) and recall (2 runs) task. Seven subjects entrained on an eight-movement sequence and eight subjects entrained on a 12-movement sequence (one subject completed both sequences in separate sessions). Learning was assessed as a combination of correct finger movements and timing (500 ms per movement) across all runs. Participants were considered "learners" if they performed at 40% accuracy or greater during recall. We used Gannett for GABA quantification relative to water. For the eight-movement sequence, four out of seven subjects were learners and for the 12-movement sequence, three out of eight participants were learners. An independent samples *t*-test was performed to examine change GABA levels from Rest1 to Recall1 between learners (n = 7) and non-learners (n = 8). Learners (M = -.71, SD = .92) had a greater change in GABA compared to non-learners (M = -.07, SD = .25) with a trending significance, p = .081. There was a significant correlation between change in GABA levels from Rest1 to Recall1 and performance accuracy of Recall1, r_s = -.47, p = .04. Older adults who were able to accurately perform the task had a more dynamic range GABA levels that correlated with performance. This modulation of GABA associated with better performance could be related to changes in neuroplasticity. Although these results are in the preliminary stages, they point to a greater understanding of aging related changes in motor performance and future rehabilitation.

Disclosures: G. Champion: None. **J.R. Nocera:** None. **L.C. Krishnamurthy:** None. **T.S. Novak:** None. **K. Mammino:** None. **K. McGregor:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.01

Topic: E.04. Voluntary Movements

Support: GB17118

Title: Conflict induced relationship between multi-unit spike trains and behavior

Authors: *J. MCDONNELL, J. S. NEIMAT, A. ALHOURANI, S. WYLIE, N. VAN WOUWE;
Neurolog. Surgery, Univ. of Louisville, Louisville, KY

Abstract: *Introduction*: Deep brain stimulation (DBS) is an effective treatment option for neurodegenerative movement disorders. In Parkinson's disease, stimulating subthalamic nuclei (STN) reduces abnormal oscillations in cortico-striatal circuits caused, presumably, by a lack of dopamine. STN activity exerts an excitatory effect on inhibitory output structures of the basal ganglia, which results in suppression of action. Previous evidence suggests functional specialization in dorsal and ventral subregions of the STN based on dissociable inputs from pre-

frontal/motor and limbic regions, respectively. More recently, DBS targeting the dorsal STN, but not the ventral STN, improved the proficiency of suppressing impulsive actions triggered in a response conflict task. In the current study, we extended this work by testing the hypothesis that neuronal spike activity in dorsal STN would increase on conflict trials that required the suppression of an incorrect action impulse. *Method*: We examined bilateral STN neuronal spike activity in 6 PD patients during performance of a Simon response conflict task. The task and recording were performed intraoperatively, prior to surgical implantation of DBS electrodes. The Simon conflict task was first performed when electrodes were situated just below the dorsal border of the STN, and a second round of trials was performed when the electrode was positioned 2-3 mm ventrally. The Simon task requires a left or right directional response to a feature (color) of a spatially lateralized stimulus. When the action signaled by the location and color of the stimulus are non-corresponding thereby inducing conflict, the inappropriate action impulse must be suppressed. *Results*: Behaviorally, we replicated the typical findings of the Simon conflict task with longer reaction times and less accurate performance on non-corresponding trials compared to corresponding trials. The preliminary neurophysiological analyses suggest there was a tendency for increased multi-unit activity in the right ventral STN with non-corresponding trials. This effect was less prevalent in dorsal and left hemisphere STN. As more data is collected, additional investigation regarding the relationship between the behavioral metric derivatives and correlating spike rates of STN subregions will be deconstructed and related to trial type, laterality, and subregion. *Conclusion*: Based on the preliminary findings, we conclude that both hemisphere and STN subregion play a role in conflict processing. In furthering our understanding of this neuroanatomical relationship, a more precision based approach can be adapted across various applications and populations.

Disclosures: J. McDonnell: None. **J.S. Neimat:** None. **A. Alhourani:** None. **S. Wylie:** None. **N. van Wouwe:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.02

Topic: E.04. Voluntary Movements

Title: The effect of limb dominance on the cortical response after mechanical perturbation to the hand

Authors: *K. HOOKS[1], K. KIANI[1], Q. FU[2];
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Abstract: The ability to move through a complex world and react to changing conditions is critical for human movement. Previous electromyography (EMG) research has shown that the

response to a mechanical perturbation on the upper extremities can be divided into three components; a short-latency reflex, long-latency response, and voluntary response (20-50ms, 50-100ms , 150+ms post-perturbation, respectively). However, the cortical dynamics underlying the corrective actions after such perturbation remains largely unknown. The focus of this research is to examine the neural contributions of the ipsilateral and contralateral hemispheres following a mechanical perturbation on either right or left hand when the hand is moving to a target. We quantified the neural activity using electroencephalography (EEG) to determine the effect of limb dominance. Right-handed subjects (n=9, 4 male, 5 female, age 25.5 yrs +/- 5.02 yrs) performed unimanual up or down targeted movements (n=200 trials) with either hand using a haptic robotic device, during which a perturbation may or may not be applied. Kinematic analysis revealed no difference between hands in the spatiotemporal characteristics of the post-perturbation movement. Furthermore, we found no difference between the cortical event-related potential (ERP) of two hands during 0 to 150 ms period post perturbation[QF1] , i.e., ERP distribution in left hand conditions mirrored the distribution in right hand conditions. Lastly, event-related spectral perturbation (ERSP) analysis of the low beta frequency band (13-20 Hz) showed stronger synchronization in contralateral motor and pre-motor areas than the ipsilateral ones during 78-122 ms post-perturbation, and this synchronization was overall significantly stronger when the dominant right hand was perturbed than the non-dominant left hand (two-way repeated measures ANOVA).

Disclosures: K. Hooks: None. **K. Kiani:** None. **Q. Fu:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.03

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R01NS120226

Title: Startling acoustic simulation causes a greater reduction in reaction time for shoulder abduction compared to hand opening

Authors: *K. WRIGHT[1], Y. ZHAO[2,1], J. P. DEWALD[1], J. YAO[1];
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Abstract: Startling acoustic stimulus (SAS) has been proven to elicit a quick release of a prepared movement, likely via the reticulospinal tract (RST). Previous research by Carlsen et al. 2009 found that finger abduction, which is thought to be mediated by corticospinal connections, does not exhibit a startle response (SR). Their results support that the SR is primarily mediated

by the RST. Following this, Honeycutt et al. 2019 found that the SR was triggered during coordinated grasp but not individuated finger movements. Based on this, they suggested that the RST is involved in whole hand coordination, but not individual finger movements.
In this study, we investigated the effect a SAS has on RT for hand opening (OPEN) compared to shoulder abduction (LIFT). Based on the findings discussed, we hypothesized that a SAS would cause a reduction in RT for both OPEN and LIFT, with a more pronounced effect for LIFT.
To test our hypothesis, we recruited four able-bodied participants with right-hand dominance, and no known hearing deficits to perform OPEN and LIFT movement tasks. OPEN and LIFT were assigned to separate trial blocks, each consisting of 25-35 trials. All trials began with an 80 dB 'ready' sound to indicate the start of the trial. Participants were instructed to self-initiate the specified movement 5-6 s after hearing the 'ready' sound. For 90% of all trials, a 2nd sound cue was delivered 1.5-2 s after the 'ready' sound to indicate participants perform the specified movement as quickly as possible. This 2nd sound was either an 80 dB 'go' sound (67% chance) or a 115 dB 'startle' sound (33% chance). EMGs were recorded from intermedia deltoid (IDL), extensor carpi radialis (ECR), and left and right sternocleidomastoid (SCM). We calculated RT as the time between the 2nd sound cue and the onset of IDL activity (for LIFT) or ERC activity (for OPEN).
Our results showed that the SAS significantly reduced the RT for both OPEN and LIFT when compared to the 'go' condition RT. However, the difference between 'startle' and 'go' RT was significantly greater for LIFT (134.2±40.5 ms) compared to OPEN (93.4±37.3 ms). As a sign for a positive startle response, SCM activity was found in 94% of SAS trials during LIFT, but only in 56% during OPEN. Additionally, we found that the SCM activity for 'startle' trials was significantly greater for LIFT compared to OPEN. These findings are consistent with the previous literature and lend additional support to the argument that the SR is mediated by RST pathways, which tend to innervate proximal muscles more heavily as compared to distal muscles.

Disclosures: K. Wright: None. **Y. Zhao:** None. **J.P. Dewald:** None. **J. Yao:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.04

Topic: E.04. Voluntary Movements

Support: MAIA project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951910

Title: Impairments of reaching corrections after rTMS over the human medial parietal cortex

Authors: *R. BREVEGLIERI, S. BORGOMANERI, A. BOSCO, M. FILIPPINI, A. TESSARI, A. AVENANTI, C. GALLETTI, P. FATTORI;
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Abstract: Human area V6A (hV6A) is located in the posteromedial part of the posterior parietal cortex and is involved in movement planning and state estimation of the arm during reaching actions. Patient studies have demonstrated that the posterior parietal cortex is also implicated in online reaching corrections. However, it is still unknown whether hV6A plays a causal role in this process. To investigate the causal role of hV6A in action reprogramming, we interfered with hV6A functions using repetitive transcranial magnetic stimulation (rTMS, 10Hz, 3 pulses) in a group of right-handed healthy participants. The position of hV6A in each participant according to the Talairach coordinates used in a previous TMS study (Ciavarro et al J Cogn Neurosci. 2013 Sep;25(9):1453-62) was assessed by frameless stereotaxic neuronavigation (Softaxic system, EMS Medical, Bologna). Participants performed visually-guided reaching movements towards targets located at different depths and directions with the arm contralateral (right) to the stimulation site (left hV6A). rTMS was delivered 50 ms after the movement onset. In a small amount of trials, the reaching target disappeared and re-appeared in a different location, so the participants had to perform in-flight adjustments of the arm trajectory. Following stimulation over hV6A, we found larger deviations of the perturbed trajectory, specifically when the perturbation required further extension of the arm. These deviations followed a specific time-course, being present in the middle of the movement and never in the final part of it. This work provides the first evidence of a functional role of hV6A in action reprogramming during reaching.

Disclosures: R. Breveglieri: None. **S. Borgomaneri:** None. **A. Bosco:** None. **M. Filippini:** None. **A. Tessari:** None. **A. Avenanti:** None. **C. Galletti:** None. **P. Fattori:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.05

Topic: E.04. Voluntary Movements

Support: Burroughs Wellcome Fund Career Award for Medical Scientists Program A133642

Title: Explicit motor sequence learning in Parkinson's Disease

Authors: *K. PRESBREY, K. LOUIE, M. YAROSHINSKY, D. WANG;
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Abstract: The neural dynamics of explicit motor sequence learning in Parkinson's Disease (PD) are poorly understood, and there are currently no good treatments for impairments in motor learning in PD patients. In either a high or low dopamine state, PD patients practiced known sequences for multiple days in a typing task that required discrete sequence production upon presentation of visual cues. Clinical motor assessments were used to verify treatment state and score acute motor impairment each day of the experiment. Chronic wireless brain implants located in the cortex and basal ganglia recorded local field potential, and a custom behavioral apparatus determined finger kinematics. In this confirmatory study, patients exhibited learning, and neural activity demonstrated learning- and treatment-specific changes.

Disclosures: K. Presbrey: None. **K. Louie:** None. **M. Yaroshinsky:** None. **D. Wang:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.06

Topic: E.04. Voluntary Movements

Title: Voluntary motor commands are preferentially released during restricted sensorimotor beta rhythm phases

Authors: *S. J. HUSSAIN[1], M. K. VOLLMER[2], I. ITURRATE[3], R. QUENTIN[4];
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Abstract: BACKGROUND: Sensory perception and memory are enhanced during restricted phases of ongoing brain rhythms, but whether voluntary movement is constrained by brain rhythm phase is not known. Voluntary movement requires motor commands to be released from the contralateral motor cortex (M1) and transmitted to spinal motoneurons and effector muscles. However, M1 activity oscillates between excitatory and inhibitory phases that correlate with single-neuron spiking rates and descending output from M1 to the spinal cord. Here, we tested the hypothesis that voluntary motor commands are preferentially released from M1 during circumscribed phases of ongoing sensorimotor rhythms. If true, contralateral but not ipsilateral sensorimotor rhythm phase during motor command release should be biased towards a specific phase. METHODS: 21 healthy adults performed a self-paced finger movement task during electroencephalography (EEG) and electromyography (EMG) recordings. During this task, subjects viewed a series of pictures on a computer screen and pressed a button with their left index finger whenever they wished to view the next picture in the series. Single-pulse TMS was used to determine each subject's corticomuscular conduction latency between M1 and the left first dorsal interosseous muscle. Then, the time of motor command release preceding each finger

movement was estimated by subtracting each subject's corticomuscular conduction latency from movement-specific EMG onset times. Finally, we calculated the contralateral and ipsilateral sensorimotor mu and beta phase angle at this time point for each finger movement. Phase angles were averaged within each subject and separately pooled across all subjects for each frequency and hemisphere. RESULTS: 31.01 ± 13.7% and 64.94 ± 19.33% of finger movements contained mu and beta periodic activity that exceeded the aperiodic component of the power spectrum. Further, contralateral beta phase angles during motor command release clustered near the peak of the beta cycle, exhibiting a significant unimodal deviation from uniformity at 122.48° (Rayleigh's test, p=0.001). In contrast, contralateral mu phase angles, ipsilateral mu phase angles, and ipsilateral beta phase angles were uniformly distributed across all possible phases during motor command release (Rayleigh's test, p>0.25 for all). CONCLUSION: Consistent with our hypothesis, results demonstrate that voluntary motor commands were preferentially released from M1 just after the peak phase of contralateral sensorimotor beta cycles. We conclude that contralateral beta phase sculpts a fundamental aspect of voluntary human movement.

Disclosures: S.J. Hussain: A. Employment/Salary (full or part-time):; University of Texas at Austin. **M.K. Vollmer:** None. **I. Iturrate:** A. Employment/Salary (full or part-time):; Amazon EU. **R. Quentin:** A. Employment/Salary (full or part-time):; Université Claude Bernard Lyon.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.07

Topic: E.04. Voluntary Movements

Support: WVCTSI Award
U54GM104942
Brain Initiative R24 MH106057-03
NIH Grant P20GM103434
NSF IIP-1929529

Title: A future for ecologically valid human neuroimaging: demonstration of real-time, ambulatory, low-dose PET imaging

Authors: *N. K. SIVA[1], C. BAUER[7], C. GLOVER[2], A. STOLIN[3], J. W. LEWIS[4], S. CHANDI[5], H. MELNICK[5], G. MORANO[3], B. PARKER[3], M. MANDICH[6], J. QI[8], S. MAJEWSKI[8], J. A. BREFCZYNSKI-LEWIS[2];
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Abstract: Background:Motion enabling human brain-dedicated PET could offer quantitative imaging with high spatial resolution of both surface and deep brain structures. Here we test the motion tolerance of a limited field-of-view prototype PET imager, AMbulabory PET (AMPET), with the goal of facilitating ecologically valid study designs and ability to scan subjects with difficulty remaining still, such as those with movement disorders, cognitive impairments, or the very young/old.
Methods:AMPET consisted of 12 silicon photomultiplier-based detectors arranged in a ring and suspended by an external support system, such that the friction-fit imager moved with the patient head. Immediately after a 1-2 millicurie bolus of F^{18}-FDG, eleven subjects (including one with a leg prosthetic, 2 removed due to data collection error) performed a 6-minute task that alternated between 30 second periods of walking-in-place and standing still. We compared motion artifact in brain vs. non-brain region of interests, and measured activity in task-appropriate (leg motor cortex, basal ganglia, thalamus) vs. non-task brain regions.
Results:A mixed effects model demonstrated no statistically significant difference in the ratio of total brain activity to activity spillover to surrounding regions for the walking and standing still periods. During image processing, we did not visually detect any blurred edges or ghosting effects that are usually indicative of motion artifacts. For task analysis, a priori leg motor cortex showed greater activity than non-motor/ non-visual-motor cortical regions ($p<0.01$). Our results were further supported by exclusively contralateral leg ROI activation in a leg amputee participant, and increased activity in basal ganglia and thalamus in a subset of 4 participants with the imager repositioned lower on the head.
Conclusion:Based on both visual and statistical measures, we found no significant motion artifacts, and task-related data were uncompromised. Furthermore, we were able to image deep brain structures, which is not possible with other motion-enabling neuroimaging modalities (EEG, fNIRS) and scanned in an ordinary laboratory without shielding, unlike MEG or MRI. The low dose enables longitudinal scanning and access to vulnerable populations. Combining motion-enabling PET with bolus-infusion delivery of radioligand could enable fMRI-like studies with minute level temporal resolution and spatial resolution within millimeters. Previously confined to highly adapted laboratory environments, real-time neuroimaging of motor functions, addiction behaviors, and social interactions could be studied in more natural settings.

Disclosures: N.K. Siva: None. **C. Bauer:** None. **C. Glover:** None. **A. Stolin:** None. **J.W. Lewis:** None. **S. Chandi:** None. **H. Melnick:** None. **G. Morano:** None. **B. Parker:** None. **M. Mandich:** None. **J. Qi:** None. **S. Majewski:** None. **J.A. Brefczynski-Lewis:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.08

Topic: E.04. Voluntary Movements

Support: WVCTSI Award U54GM104942
Brain Initiative R24 MH106057
NIH Grant P20GM103434
NSF IP-1929529

Title: Brain imaging in motion: Bolus-infusion task-based FDG-PET imaging of human brain during treadmill walking

Authors: *C. GLOVER[1], N. SIVA[1], A. STOLIN[2], G. MORANO[2], A. HAHN[4], L. RISCHKA[5], R. LANZENBERGER[5], S. MELROY[3], H. MELNICK[3], K. RADCLIFF[1], S. CHANDI[1], T. WUEST[3], S. MAJEWSKI[6], J. A. BREFCZYNSKI-LEWIS[3];
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Abstract: Background: While Positron Emission Tomography (PET) provides a successful method of quantifying neuronal activation in the brain, few devices allow the flexibility to image freely moving subjects. Also, most PET paradigms utilize radioligands such as the widely available metabolic marker 18F-FDG, as a bolus injection, which requires separate day visits for task vs. baseline. The present study examines a novel support system for a wearable limited field-of-view prototype PET imager, Ambulatory PET (AMPET), in combination with radioligand delivery protocols to allow for task-based imaging similar to block-design fMRI.
Methods: AMPET consists of a ring of 12 silicon photomultiplier-based detectors friction-fit to follow the patient's head movements. The Biodex Unweighing System was adapted to not only support the weight of the patient in the event of a fall, but to fully support the PET imager with a counterweight. The support system allowed the participant to walk naturally on a treadmill while wearing the imager. An EMG (muscle activity) and VICON (infrared motion detection) study (n=20) was performed with a facsimile weighted imager to test for harmful muscle activity in the neck or back and motion interference during walking movements. For 18F-FDG imager study, a bolus-infusion 18F-FDG protocol was used for a 20-minute task alternating between 5 minute intervals of walking and seated (rest). A bolus of 1.1 mCi 18F-FDG was delivered followed by a constant infusion of 0.9 mCi for the entire duration of the imaging session (n=5, 5 additional participants had a rate recording error). Analysis used an a priori leg motor cortex ROI based on a previous PET walking study (Huang et al. 2018), comparing brain activation patterns during walking and rest.
Results: The EMG study of the facsimile imager supported by the Biodex system revealed no significant increase of muscle activity for walking task. VICON data showed speed and acceleration movements were impaired (speed $t=9.2$, $p<.0001$, acceleration $t=8.2$, $p<0001$). In the AMPET imaging study, our limited field of view imager was able to capture the expected trend of higher activity in leg motor ROI in the walking period (normalized mean±sd = 0.0041±0.0008) as compared to rest (0.0039±0.0009)
Conclusion: The task-based 18F-FDG motion-enabling AMPET may allow multiple tasks and baseline scans in a single session of behaviors that require moderate motion, but not fast accelerations. Additionally, our prototype imager will allow for an advancement in neuroimaging

by permitting real-world and virtual immersive studies with free range of motion to be conducted. A higher dose of ligand is likely needed for superior image quality.

Disclosures: C. Glover: None. **N. Siva:** None. **A. Stolin:** None. **G. Morano:** None. **A. Hahn:** None. **L. Rischka:** None. **R. Lanzenberger:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Siemens Healthcare. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BM Health GmbH. F. Consulting Fees (e.g., advisory boards); Shire. **S. Melroy:** None. **H. Melnick:** None. **K. Radcliff:** None. **S. Chandi:** None. **T. Wuest:** None. **S. Majewski:** None. **J.A. Brefczynski-Lewis:** None.

Poster

P535. Cortical Planning and Execution: Human Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P535.09

Topic: E.04. Voluntary Movements

Title: Machine learning of EEG data during task visualization can distinguish between well-learned and unfamiliar motor skills

Authors: *J. SHATTUCK[1], M. R. JOHNSON[2];
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Abstract: Neural activity that occurs during imagined performance of a motor task (motor imagery) has been well established to be neurophysiologically similar to the actual performance of the motor task, albeit greatly attenuated. Machine learning classification of different visualized tasks can differentiate sleeping from drumming, listening to music or walking, etc. However, determining the skill level of the subjects visualizing the task has not been explored with this method. EEG data collected during visualization of a Known (previously acquired) skill were compared to data collected during visualization of an Unknown (non-acquired) motor skill, and analyzed with a relatively simple convolutional neural network. Our hypothesis was that a binary (Known or Unknown) skill level of the imagined task could thus be classified from the EEG data.Twenty human subjects were instructed to visualize performing a motor-based task from a first-person perspective for 2 minutes with eyes open in a quiet room. Each subject wore a 14 channel, 128 Hz portable EEG headset. Prior to the session, each subject chose two repetitive motor sequence skills (juggling, drumming or similar repetitive motion): one in which they were proficient, and the other in which they were familiar, but not at all proficient. The order of the known or unknown skill visualization was randomized across subjects.A 14x3 matrix representing the theta/alpha/beta power at each of the 14 electrodes was analyzed to detect whether the subject was visualizing a Known or Unknown skill. Data from all participants was fed into a neural network consisting of one convolutional layer and one fully connected layer,

using the DeLINEATE deep learning analysis toolbox. 70% of the data was used for training, 15% for validation, and 15% for testing, and the analysis was repeated 20 times with different random training/validation/testing splits.Average test accuracy was 73.5% (relative to a chance level of 50%). This finding suggests the potential for an artificial neural network to detect neural patterns of differences that may represent encoded motor memories via EEG during visualization of a motor sequence task.Next steps include analyzing subsets/regions of electrodes to determine with greater precision the neural substrates of such motor memories, and potentially refining our methods to achieve greater accuracy. Additional studies on subjects with varying degrees of proficiency on the same skill could offer further insights to the acquisition and storage of motor skill.

Disclosures: J. Shattuck: None. **M.R. Johnson:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.01

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Novo Nordisk Foundation Laureate Program
Independent Research Foundation Denmark

Title: A spinal microcircuit for muscle activation sequences

Authors: *C. BELLARDITA, M. MARCANTONI, O. KIEHN;
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Abstract: Movements as swimming and walking require a complex sequence of muscle contractions along the body. The spinal cord is the final executor of the neural code translating descending motor commands and sensory feedback into detailed and coherent patterns of muscle activity. Here, we examine how a spinal circuitry for multi-joint movements - where the V2a neurons represent the core element - converts a general motor command into detailed muscle activity for functional movements. Combining mouse intersectional genetics with *in vivo* optogenetic and electrophysiology in adult anesthetized mice, we show that short optical activation of V2a spinal neurons along the spinal cord activate discrete muscles in an ordered fashion, giving rise to a topographic map similar to the muscolotopic map formed by the motor pools. Connectivity profiles of segmentally defined V2a neurons and simultaneous electrophysiological recordings of opto-identified V2a neurons with multiple muscles revealed that V2a neurons make distinct spinal microcircuits that enable simultaneous recruitment of multiple muscles. V2a neurons form a feedforward descending excitatory system in the spinal cord that together with sensory afferents, motor neurons and muscles form functional units.

Prolonged optical activation of V2a neurons in a single unit recruit multiple muscles leading to a functional movement. In contrast, targeted i*n vivo* silencing of segmentally defined V2a neurons heavily perturbed coordinated muscle activity along the animal body in complex motor tasks as swimming. These data identify the neuronal substrate and the functional principles of a spinal circuit involved in constructing muscle activation sequences and provide the logic on how spinal circuitries sculpt muscle action over time and space for meaningful motor behaviors.

Disclosures: C. Bellardita: None. **M. Marcantoni:** None. **O. Kiehn:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.02

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR

Title: Excitatory spinal v3 interneuron subpopulations diversify across hierarchical temporal and spatial developmental pathways

Authors: *D. DESKA-GAUTHIER[1], H. ZHANG[2], J. BOROWSKA[1], C. JONES[1], C. MACKAY[3], Y. ZHANG[3];
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Abstract: Neural circuits in the spinal cord are comprised of vastly heterogeneous interneurons required for appropriate execution of movement. Discrete spinal interneuron classes emerge during early embryogenesis, although by postnatal stages extensive subpopulation diversity exists within each class. While combinatorial transcription factor profiles reveal distinct molecular IN clusters, how this molecular logic is acquired and to what extent it translates into functional distinctions between spinal IN subpopulations remains largely unknown. In the current work, through a focus on the cardinal V3 interneuron class, we reveal hierarchical early embryonic temporal-spatial mechanisms underlying molecular subpopulation diversification in the mouse spinal cord. First, neurogenesis timing separates V3 interneurons into either early-born or late-born temporal subclasses upon cell-cycle exit. Second, post-mitotic spatial mechanisms further diversify V3 INs within each temporal subclass into molecularly, anatomically, morphologically, and electrophysiologically distinct subpopulations. Thus, our work reveals early embryonic temporal and spatial mechanisms as key developmental logic for the diversification of spinal interneurons into functionally distinct subpopulations.

Disclosures: D. Deska-Gauthier: None. **H. Zhang:** None. **J. Borowska:** None. **C. Jones:** None. **C. Mackay:** None. **Y. Zhang:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.03

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Howard Hughes Medical Institute

Title: Voltage imaging in vivo shows that V3 neurons modulate swim strength

Authors: *U. L. BÖHM[1], Y. KIMURA[3], T. KAWASHIMA[4], M. B. AHRENS[5], S.-I. HIGASHIJIMA[6], F. ENGERT[2], A. E. COHEN[1];
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Abstract: Motor systems must constantly adapt their output to maintain a desired trajectory. While the basic mechanisms underlying the generation of rhythmic locomotion in the spinal cord are well described, little is known about how the network modulates its output strength. A major challenge has been the difficulty of recording from spinal neurons during behavior. Here, we use voltage imaging to map the membrane potential of glutamatergic neurons throughout the spinal cord of the larval zebrafish during fictive swimming in a virtual environment. We mapped the spiking, subthreshold dynamics, relative timing and sub-cellular electrical propagation across large populations of simultaneously recorded cells. We identified a sub-population of tonic-spiking ventral V3 neurons whose spike rate correlated with swimming strength. Optogenetic activation of V3 neurons led to stronger swimming. Genetic ablation of V3 neurons led to reduced locomotor adaptation. Our study leverages the power of voltage imaging to identify V3 neurons as a critical driver of motor adaptation in zebrafish.

Disclosures: U.L. Böhm: None. **Y. Kimura:** None. **T. Kawashima:** None. **M.B. Ahrens:** None. **S. Higashijima:** None. **F. Engert:** None. **A.E. Cohen:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.04

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH R01 DC016413
PEW Scholar Award (M.W.B.)
McKnight Scholar award (M.W.B.)
McDonnell Center for Cellular and Molecular Neurobiology at Washington University (M.S.)

Title: Mapping the structure of ipsilateral inhibition in axial motor circuits

Authors: *M. SENGUPTA, M. W. BAGNALL;
Washington University, Sch. of Med., Saint Louis, MO

Abstract: The spinal cord is responsible for generating motor output. Traditionally, the field has emphasized the role of commissural inhibition that alternates with excitation in motor neurons, setting up a phase difference vital for locomotion. Ipsilateral inhibition, however, remains much less explored. Two major ipsilateral inhibitory populations in the spinal cord are the V1 (En1+) and V2b (GATA3+) classes. So far, these have been implicated in flexor-extensor alternation in limb circuits where they display a complementary, reciprocally inhibitory function. How these neurons govern axial (body) circuits, especially in the rostro-caudal (R-C) axis is still not known. To investigate whether these neurons are important in R-C coordination, we used larval zebrafish to generate a broad map of connectivity, from V1 and V2b neurons to both motor and sensory targets, using *in vivo* optogenetics and electrophysiology. V1 neurons send ascending axons towards the brainstem while V2bs send descending axons caudally. Both V1 and V2b neurons exhibit long axons running approximately 5-7 segments, but surprisingly, their postsynaptic connectivity was highly asymmetric and unique along the rostro-caudal axis. Despite projecting long axons (7+ segments), V1 neurons contacted fast and slow motor neurons only locally (< 3 segments). Premotor targets like excitatory V2a population, V2bs, and commissural neurons also received only short range V1 inhibition. In contrast, V2b neurons robustly inhibited motor and premotor targets including V1 neurons distally up to 7 segments. On the sensory front, V1 and V2b neurons also exhibited selective connectivity. Unlike its motor contacts, V1 neurons strongly inhibited Commissural Primary Ascending neurons at short and long distances indicating an important sensory gating function for V1s. V2b neurons, on the other hand, did not directly inhibit either of the two sensory targets tested implying a primarily motor related role. To test the functional implications of this organization, we built a reduced model of the unilateral spinal cord and showed that the local pattern of V1 inhibition is crucial for motor burst termination and smooth propagation of the locomotor wave. Current work is aimed at understanding the effects of V2b inhibition and how these two sources are integrated for R-C coordination. Collectively, these data show that ipsilateral inhibition is asymmetric in the R-C axis of the spinal cord in two distinct ways: V1 and V2b neurons inhibit different target populations; and they exhibit unique patterns of connectivity in the R-C axis, suggesting multiple, distinct functions of ipsilateral inhibition in motor control.

Disclosures: M. Sengupta: None. **M.W. Bagnall:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.05

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 NS047357

Title: Loss of Foxp2-V1 interneurons results in hyper flexion and limb discoordination during locomotion.

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Abstract: In tetrapod animals, coordinated movement requires the orchestration of motor neuron firing that directs muscle contraction across multiple joints and limbs, thereby achieving changes in desired trajectory upon volitional commands or environmental perturbations. Such patterned motor output depends on the activity of spinal premotor interneurons. For example, flexor-extensor alternation within an individual limb relies on ipsilaterally projecting inhibitory interneurons that reciprocally inhibit flexor and extensor spinal kernels and flexor and extensor motor neurons. The largest group of spinal inhibitory interneurons with ipsilateral projecting axons are V1 interneurons, a molecularly heterogenous population that can be subdivided into at least four major clades on the basis of differential transcription factor expression. The largest of these clades is defined by expression of the Forkhead box protein P2 (Foxp2) transcription factor. Prior work has suggested that this subset exhibits characteristics reminiscent of reciprocal Ia inhibitory interneurons (IaINs) responsible for flexor/extensor coordination. To characterize the role of Foxp2-V1 interneurons during motor behaviors, we took advantage of an intersectional genetic strategy in which *En1-Cre; Foxp2-Flpo* mice were crossed with *RC-PFtox* mice, resulting in silencing of FoxP2-V1 interneurons due to expression of tetanus neurotoxin. These mice show a strong ataxic phenotype at age P5-P6, and adult mice cannot follow normal walking treadmill speeds. These motor deficits likely result from silencing Foxp1/En1 neurons in multiple systems distributed throughout the neuroaxis, including cerebellar, midbrain locomotor, dopaminergic nigral, and spinal systems. Kinematic analyses of treadmill walking in mutant animals revealed over-flexion throughout the step cycle, mainly at the hip and knee joints, consistent with prior descriptions of hyperflexion upon V1 ablation. To isolate deficits specific to spinal circuits, P7-P8 mutant mice were analyzed after L-DOPA induced air stepping. These pups demonstrate a lack of intra and interlimb coordination and, strikingly, coactivation of EMG activity of ankle flexor and extensor muscles, contrasting with normal reciprocal muscle activation, and coordination between different joints across all four limbs in wild-type mice. Our

results suggest that Foxp2-V1 interneurons are involved in flexor-extensor activation and interlimb coordination, both functions suggested in the past to be contributed by IaINs.

Disclosures: O.C. Mistretta: None. **A.E. Worthy:** None. **N.M. Greenfield:** None. **J.B. Bikoff:** None. **A.W. English:** None. **F.J. Alvarez:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.06

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF NeuroNex 2015317
NSF IIS160811

Title: Analyzing the performance of central pattern generators for a rat synthetic nervous system

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Abstract: This work investigates how neural parameters and external inputs affect the performance of a central pattern generator (CPG) used to model mammalian locomotion. In our previous work, when designing a synthetic nervous system, choices of neural parameters were determined using genetic algorithms combined with hand-tuning. However, the impact that different intrinsic parameters have on changing the behavior of the neural controller is not fully understood. In particular, little is known about how weak mutual excitation between oscillators alters output. This hypothesized connection is based on the observation from several groups that synchronous rhythmic excitation of flexor and extensor motor neurons occur when V1 and V2b inhibition is absent. When designing a model of the CPG, the oscillation frequency is the most essential part, and a desired frequency can be attained by various combinations of neural parameters. Including the weak mutual excitation into the system limits the operation range of the CPG (i.e., it shrinks the phase orbit size in the phase plane). Another aspect to consider when designing a model of the CPG is its reaction to external stimuli or perturbations. Our results show that for CPGs oscillating at the same frequency, the external drive makes the system less sensitive to external inputs, whereas the weak connection does not change the phase response curve of the system. This study provides insights for studies of the locomotion CPG, and will benefit the design of bio-robotic controllers.

Disclosures: K. Deng: None. **A.J. Hunt:** None. **H.J. Chiel:** None. **R.D. Quinn:** None.

Poster

P565. Motor Systems: Connectivity of CPGS

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P565.07

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH grant MH117808

Title: A seventeen lab collaboration to reconstruct the connectome of the adult Drosophila ventral nerve cord

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Abstract: The invertebrate ventral nerve cord (VNC), homologous to the vertebrate spinal cord, integrates sensory signals from the body with commands from the brain in order to control movements. Mapping the morphology and connectivity of each of the VNC's neurons is a crucial step toward understanding and reverse-engineering animals' motor control algorithms. We recently reported a synapse-resolution electron microscopy (EM) dataset of the VNC of an adult female fruit fly, a species that can flexibly and precisely coordinate its six legs and two wings to accomplish a wide variety of behaviors. Here, we automatically segmented cells and predicted synaptic partners across the entire EM dataset to accelerate reconstruction of the ~15,000 neurons in the adult fruit fly's VNC. Manual proofreading of the reconstructed neurons is often necessary, but this takes between a few minutes to an hour per neuron. At this speed, proofreading particular neurons-of-interest and their upstream and downstream partners can be accomplished in days to weeks. To facilitate the community's access to this dataset, we oversee a collaborative effort where members of seventeen (and counting) different labs are proofreading distinct circuits. Importantly, this open collaboration provided opportunities for remote work for students and researchers affected by lab shutdowns brought on by the pandemic. All proofreading occurs within one unified dataset, so each lab's efforts contribute to the broader goal of obtaining a fully proofread VNC connectome. Furthermore, we are building computational tools to allow users to easily annotate and query the connectome data, facilitating exploration and analysis even by users with limited programming experience. This collaborative approach to connectome proofreading is bringing VNC researchers together behind a common goal, while allowing each researcher to quickly map the structure and connectivity of the neurons they care most about.

Disclosures: J.S. Phelps: None. **R. Lu:** A. Employment/Salary (full or part-time):; Zetta AI. **N. Kenmitz:** A. Employment/Salary (full or part-time):; Zetta AI. **K. Lee:** A. Employment/Salary (full or part-time):; Zetta AI. **B. Mark:** None. **A. Halageri:** A. Employment/Salary (full or part-time):; Zetta AI. **M. Castro:** A. Employment/Salary (full or part-time):; Zetta AI. **D. Ih:** A. Employment/Salary (full or part-time):; Zetta AI. **M. Tammam:** A. Employment/Salary (full or part-time):; Zetta AI. **S. Gerhard:** A. Employment/Salary (full or part-time):; UniDesign Solutions GmbH. **S. Dorkenwald:** None. **F.C. Collman:** None. **C.M. Schneider-Mizell:** None. **D. Brittain:** None. **C.S. Jordan:** None. **T. Macrina:** A. Employment/Salary (full or part-time):; Zetta AI. **J.C. Tuthill:** None. **H. Seung:** A. Employment/Salary (full or part-time):; Zetta AI. **W.A. Lee:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.01

Topic: F.05. Neuroimmunology

Support: NIH/NIGMS

Title: Electrical stimulation of the dorsal motor nucleus of the vagus activates the efferent arm of the inflammatory reflex shifting the cytokine balance in murine endotoxemia towards suppression of inflammation

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Abstract: The inflammatory reflex is a vagus nerve-mediated and brainstem-integrated physiological mechanism that regulates immunity and inflammation. The efferent vagus nerve originates in brainstem nuclei, including the dorsal motor nucleus of the vagus (DMN). We have shown that optogenetic stimulation of cholinergic DMN neurons suppresses serum TNF levels during endotoxemia *(PNAS, 2020, 117(47):29803*). While the clinical translation of optogenetics for brain stimulation remains challenging, electrical stimulation is widely used in bioelectronic therapeutic approaches targeting the brain. However, the efficacy of altering cytokine levels using electrical DMN stimulation remained unexplored. To provide insight, we studied the effect of electrical DMN stimulation on serum pro- and anti-inflammatory cytokines in murine endotoxemia. Electrical DMN stimulation was performed in male 10-12 week old C57BL/6 mice, using a stereotaxic-guided approach. Mice were anesthetized using isoflurane and the head was positioned on a stereotaxic frame for a surgical intervention to insert a concentric bipolar

electrode into the left DMN according to Paxinos and Franklin mouse brain atlas coordinates. Electrical stimulation (50 μA, 30 Hz, 260 μs) or sham-stimulation was performed for five mins, followed by lipopolysaccharide (LPS, 0.5 mg/kg) i.p. administration. Mice were euthanized 90 mins after LPS and blood (via cardiac puncture) was collected and processed for pro- and anti-inflammatory cytokine analysis. The brain was collected for histological analysis following cardiac perfusion. Electrical DMN stimulation significantly decreased serum levels of the pro-inflammatory cytokine TNF compared with sham stimulation (1,581 ± 465 pg/ml vs 1,139 ± 357 pg/ml, n = 15 per group, P=0.0069). Electrical DMN stimulation also enhanced serum levels of the anti-inflammatory cytokine IL-10 (9,433 ± 1,491 pg/ml vs 14,784 ± 1,758 pg/ml, P=0.0284). Brainstem histological analysis, including immunostaining for choline acetyltransferase (ChAT) and confocal imaging verified the accurate positioning of the stimulating electrode in the left DMN ChAT-positive neurons - a major source of efferent vagus nerve cholinergic axons. These results indicate for the first time that the efferent arm of the inflammatory reflex can be activated by DMN electrical stimulation shifting the cytokine balance towards suppression of inflammation. These findings may enable future clinical bioelectronic approaches targeting DMN for therapeutic benefit in conditions characterised by excessive inflammation. This study was funded in part by NIH/NIGMS.

Disclosures: A. Falvey: None. **S. Palandira:** None. **Q. Zeng:** None. **R. Stojchevski:** None. **D. Avtanski:** None. **K.J. Tracey:** None. **V.A. Pavlov:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.02

Topic: F.05. Neuroimmunology

Support: NIH Grant 5P01AI073693
DOD Grant W81XWH1910759

Title: Disrupted neural encoding of immune signals in vagus nerve after sepsis

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Abstract: Studying the long-term effects of sepsis has become an increasingly important issue. As more patients survive sepsis, it has become obvious that the nervous system is a major target for the post-sepsis syndrome. Recent work has suggested that a change in vagus nerve (VN) activity may play a role in the immunological changes that take place after sepsis. In healthy mice, recordings from the VN have shown that it can encode immune-related signals, such as cytokines. The goal of this study is to determine whether the VN is altered by sepsis such that the

cytokine-specific neurograms are not correctly encoded. Sepsis was modeled with the cecal-ligation-and-puncture method in male C57BL/6 mice. After a recovery period of 4 weeks, VN recordings were performed in 16 Sham and 21 sepsis-surviving (*Sep*) mice, under isoflurane anesthesia, using cuff electrodes (CorTec, Freiburg, Germany). Recordings consisted of a baseline (BL) period (0-20 min) followed by intra-peritoneal injections of TNF (20-40 min) and IL-1β (40-60 min). In other experiments, IL-1β was given before TNF. We measured the compound action potentials (CAPs) carried by the VN axons. For each recording, we inputted the CAP frequencies at each sec into Bayesian decoding algorithms to estimate the probability that each time-point belonged to one of 3 classes: BL, TNF or IL-1β. We then compared the probabilities from the algorithm to the actual class. During BL, sepsis survivors have elevated baseline CAP frequency when compared to controls (Fig. 1A, Sham = 2.7±0.6 CAPs/s, *Sep* = 14.8±2.1 CAPs/s, $p = 5x10^{-7}$, t test). Crucially, the cytokines trigger a robust increase of CAP frequency in Sham mice (Fig. 1A, 5.8±0.8 CAPs/s, $p = 3x10^{-5}$ vs. Sham at BL, t test), whereas sepsis survivors do not change their activity after cytokine injection (Fig. 1A, *Sep* = 14.1±1.6 CAPs/s, p = 0.6 vs. *Sep* at BL, t test). The probability of predicting the correct class is higher for controls at BL (Fig. 1B; Sham = 80±5%, *Sep* = 59±5%, p = 0.006, t test) and TNF (Fig. 1B, Sham = 78±5%, *Sep* = 62±5%, p = 0.046, t test), but not significantly different after IL-1β (Fig. 1B, Sham = 81±4%, *Sep* = 73±4%, p = 0.18, t test). We show that sepsis leads to long-term changes in the function of the VN which can be directly measured using electrophysiological techniques. Sepsis survivors display a higher level of activity in the VN, reflecting a deviation from the homeostatic state. When cytokines are injected into sepsis survivors, the VN does not respond with the expected increase in activity that occurs in control mice. Our data indicate that sepsis disrupts the neural code in the VN, which likely contributes to the dysregulated brain-immune axis that is part of the post-sepsis syndrome.

Disclosures: J.J. Strohl: None. **P.T. Huerta:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.03

Topic: F.05. Neuroimmunology

Support: This study was funded in part by NIH/NIGMS.

Title: Transient Receptor Potential Ankyrin 1 (TRPA1) mediates afferent signals in the inflammatory reflex

Authors: *H. A. SILVERMAN[1], T. S. HUERTA[1,2], E. H. CHANG[1,2], A. CHEN[1], K. J. TRACEY[1,2,3], S. S. CHAVAN[1];
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Barbara Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY; [3]Elmezzi Grad. Sch. of Mol. Med., Manhasset, NY

Abstract: The inflammatory reflex is a physiological mechanism in which afferent vagus nerve signaling activated by inflammatory mediators, is functionally associated with efferent vagus nerve-mediated output to regulate proinflammatory cytokine production and inflammation. Efferent signals in the vagus nerve terminate onto acetylcholine-producing T cell subset. Acetylcholine regulates cytokines production by interacting with alpha-7 nicotinic receptor (α7nAChR) expressed on monocytes and macrophages. Despite this extensive understanding of the efferent pathway, the afferent mechanism of the inflammatory reflex is less defined. Here we show that transient receptor potential ankyrin 1 (TRPA1) in sensory vagus neurons is sufficient to activate the inflammatory reflex and regulate cytokine production. Vagus TRPA1 was selectively activated using optopharmacology. Optovin, a selective TRPA1-ligand was placed directly on the vagus nerve prior to stimulation with 405nm light. Selective activation of the vagus nerve TRPA1 using optopharmacology stimulates the inflammatory reflex to inhibit cytokine responses to bacterial lipopolysaccharide in wild type mice (sham: 920.9 ± 87.9 pg/ml, n=9 vs. stimulated: 456.8 ± 69.4 pg/ml, n=10; Welch's T-test $p<0.001$), but no significant differences are observed in TRPA1 knock out mice (sham: 861.0±91.9 pg/ml, n=9 vs. stimulated: 795.3±92.8pg/ml, n=8; Welch's T-test p=0.622). The TRPA1-mediated cytokine reduction in wild type mice is ablated by rostral, but not by caudal vagotomy, indicating that the signals are afferent in nature (rostral n=12 vs. caudal n=9: 998.7.9±57.5 pg/ml, 794.8±49.7 pg/ml; Welch's T-test $p<0.05$). In contrast to its TNF suppressive effect in wild type mice, activation of TRPA1-signals failed to inhibit TNF levels in α7nAChR knockout mice during endotoxemia (sham: 2132±390.6pg/ml, n=10 vs. stimulated: 1562±250.5pg/ml, n=12; Welch's T-test p=.237). Taken together these results indicate that targeted stimulation of TRPA1 on afferent vagus nerve fibers is sufficient to activate the sensory arc of the inflammatory reflex and identify a potential therapeutic target for inflammation.

Disclosures: H.A. Silverman: None. **T.S. Huerta:** None. **E.H. Chang:** None. **A. Chen:** None. **K.J. Tracey:** None. **S.S. Chavan:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.04

Topic: F.05. Neuroimmunology

Support: NIH
NIGMS

Title: Sensory neurons of the vagus nerve respond to inflammatory mediators

Authors: *T. S. HUERTA[1,2], B. HAIDER[1], S. S. CHAVAN[1,2,3], K. J. TRACEY[1,2,3], E. H. CHANG[1,2,3];
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Abstract: Neural reflex circuits regulate inflammation and are key to maintaining homeostasis. The inflammatory reflex regulates systemic inflammation through vagus nerve signaling. While the motor efferent arm of this reflex has been described, the role of sensory neurons encoding immune mediators, and transmitting these signals to the brainstem remains unclear. Using vagus nerve recordings, our group has shown that the sensory arm of the vagus nerve transmits information linked to specific cytokines, however these recordings do not show activity at the single neuron level. Here, we use *in vivo* calcium imaging to monitor the neural activity of individual vagal sensory neurons in response to specific inflammatory mediators. We used mice that express a calcium sensor in glutamatergic neurons. Under isoflurane anesthesia, the nodose ganglion was imaged *in situ* using a 1-photon miniature microscope (Miniscope) while the inflammatory mediators tumor necrosis factor (TNF), interleukin 1β (IL-1β), or lipopolysaccharide (LPS) were applied directly to the vagus nerve. The recorded data was analyzed with a Python-based calcium imaging analysis (CaImAn) pipeline, which output calcium traces (change in fluorescence: ΔF) of individual neurons. Our results reveal that specific neurons respond to specific immune mediators. An average of 69±16.3 neurons were recorded per 1 hour imaging session. When exposed to both cytokines TNF and IL-1β, certain neurons were found to only respond to one cytokine (TNF: 23.6±10.4%; IL-1β: 19.75±15.7%), while a subset of neurons responded to both cytokines (TNF & IL-1β: 13±10.3%). IL-1β-responsive neurons had a significantly higher peak response than TNF-responsive cells (IL-1β *vs*. TNF, $p < 0.01$). This result may indicate that sensory neurons in the nodose ganglion respond differentially to specific cytokines, possibly through specific cytokine receptor binding. We also found that specific neurons were responsive to LPS. LPS-responsive neurons were found to have significantly higher peak response than the cytokine responsive neurons (LPS *vs.* TNF, $p < 0.05$), which suggests differential encoding process between cytokine receptors and potential toll-like receptor-dependent activation. These findings are the first to reveal individual vagal sensory neurons encoding information about distinct inflammatory stimuli. Further investigation may reveal fundamental principles by which the vagus nerve transmits information about inflammation to the brain. Better understanding of the neural sensing of inflammation may elucidate novel cellular targets for the treatment of inflammatory disorders.

Disclosures: T.S. Huerta: None. **B. Haider:** None. **S.S. Chavan:** None. **K.J. Tracey:** None. **E.H. Chang:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.05

Topic: F.05. Neuroimmunology

Support: NIH
NIGMS

Title: Optogenetic cholinergic stimulation of the celiac-superior mesenteric ganglia complex attenuates inflammation

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Abstract: The vagus nerve-based inflammatory reflex is an important physiological regulator of immunity and inflammation. In the efferent arm of the inflammatory reflex, vagus nerve cholinergic axonal endings interact with splenic nerve catecholaminergic neurons in the celiac-superior mesenteric ganglion (CSMG) complex (*Proc Natl Acad Sci USA, 2020, 117(47):29803). S*tudies using electrical and optogenetic neural stimulation have shown that vagus nerve cholinergic signaling controls pro-inflammatory cytokine levels. However, the role of the CSMG complex in the regulation of inflammation remained enigmatic. To provide insight, we examined the effect of selective optogenetic stimulation of cholinergic axonal endings in the CSMG complex on cytokine levels in murine endotoxemia. Transgenic female 10–14week-old mice expressing channelrhodopsin-2 on cholinergic, choline acetyltransferase (ChAT)-positive neurons - ChAT-ChR2 mice, were used in the experiments. Under isoflurane anaesthesia, the CSMG complex was located ventral and to the left of the abdominal aorta, along the splenic artery, superomedial to the left kidney. Blue laser light stimulation at 473 nm was delivered in square pulses for 10 mins at 20 Hz, 25% duty cycle via an optic fiber that was placed in contact with the CSMG complex. 30 mins following optogenetic stimulation or sham stimulation lipopolysaccharide (0.25 mg/kg) was injected i.p. and 90 mins later, mice were euthanized and blood, and spleen were collected and processed for analysis. Optogenetic stimulation of the CSMG complex in ChAT-ChR2 mice (n=14) significantly supressed serum pro-inflammatory cytokines and chemokines compared with sham stimulation (n=13), including TNF (401.7 ± 102.9 pg/ml vs 766.1 ± 207.2 pg/ml; $P<0.0001$) and MIP-1α (6,523 ± 3,041 pg/ml vs 10,672 ± 3,915 pg/ml, $P=0.0066$). Optogenetic stimulation also significantly increased the serum levels of the anti-inflammatory cytokine IL-10 levels compared with sham (30,779 ± 10,035 pg/ml vs 22,979 ± 9,669 pg/ml, $P=0.0427$). There was also a significant reduction in splenic TNF levels in the stimulated vs the sham group ($P=0.0023$). In conclusion, these results provide the first experimental evidence that selective simulation of cholinergic neuronal endings in the CSMG complex alters cytokine levels. These findings and further studies examining the specific contribution of vagal and sympathetic preganglionic cholinergic signaling will be instrumental for advancing our understanding of the role of the CSMG complex in the regulation of immune

responses, and of interest for developing new anti-inflammatory approaches. This study was funded in part by NIH/NIGMS.

Disclosures: S. Palandira: None. **A. Falvey:** None. **Q. Zeng:** None. **D.B. Avtanski:** None. **R. Stojchevski:** None. **L. Poretsky:** None. **K.J. Tracey:** None. **V.A. Pavlov:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.06

Topic: F.05. Neuroimmunology

Support: 5R01MH117004-02

Title: 5ht1ar deficient female mice exhibit altered neutrophil maturation dynamics and immune response during pregnancy

Authors: *A. NABILA[1], R. J. CHEN[1], J. G. TOTH[2], M. TOTH[2];
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Abstract: Serotonin-1A receptor (5HT1A-R) is a pre- and post-synaptically expressed receptor that has been implicated in anxiety and depression. Activation of 5HT1A-R modulates the action of anxiolytic, antidepressant, and antipsychotic medications in the central nervous system (CNS). Consistent with these clinical data, our lab has previously reported that 5HT1A-R heterozygous (H) and null (KO) mice on the outbred Swiss Webster (SW) background display an anxiety-like phenotype on the elevated plus maze. Surprisingly, despite the lack or minimal expression of 5HT1A-R outside the CNS, we observed immunological abnormalities in the genetically 5HT1A-R deficient mice. Specifically, H (and KO) females but not males exhibited adulthood cytopenia in three hemopoietic lineages (lymphocytes, platelets, and reticulocytes). Both the anxiety and the cytopenia phenotypes were transmitted to the F1 WT offspring suggesting that once initiated by a genetic receptor deficit, the phenotypes can propagate through the maternal lineage to the next generation via non-genetic transmission. So, cytopenia in H mothers may arise due to CNS 5HT1AR-mediated regulation of the peripheral immune system; however, in the F1 offspring, maternal immunological abnormalities can lead to immune activation that alters fetal development via the placenta.To better understand the immunological consequences of the 5HT1A-R deficit and its intergenerational transmission, we used single cell RNA-sequencing (scRNA-seq) to profile peripheral leukocytes from H and SW WT mothers at gestational day 10.5. We find that neutrophils in H mothers display an upregulation in immune response genes including S100a8 and Ifitm6. Furthermore, we observed a greater proportion of immature neutrophils in H mothers which may indicate an increased release of maturing neutrophils from the bone marrow to the periphery. Our results suggest that not only are neutrophil maturation and

release altered but there is an increased gestational immune response in H mothers that may lead to an inflammatory state that regulates gestational maternal-fetal communication. Overall, we report, for the first time, of immunological consequences of a deficit in 5HT1A-R in mice.

Disclosures: A. Nabila: None. **R.J. Chen:** None. **J.G. Toth:** None. **M. Toth:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.07

Topic: F.05. Neuroimmunology

Support: CONACyT CVU 695673
UNAM DGAPA IG-201321
CONACyT Frontera 2017-1802
CONACyT QUEBEC 279293

Title: Adrenal gland regulates acute inflammation and glycemia in an acute endotoxin challenge.

Authors: *E. SANTACRUZ-MARTÍNEZ, E. SOTO-TINOCO, R. BUIJS;
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Abstract: After an inflammatory event there are many changes in physiology. Among those is a modification of the immune system in a negative feedback manner as well as sympathetic driven parameters such as glycemia and heart rate, all of which are regulated by the nervous system. Currently, it is accepted that the immune system communicates its state of activation through cytokines. Nevertheless, the nervous system shows signs of activation before these proteins can be secreted indicating the presence of other pathways of activation. Prostaglandins are known to be immune lipidic mediators that are secreted prior to cytokines. To test whether prostaglandin could participate un this early response, its synthesis was inhibited pharmacologically in endotoxin challenged rats. Neuronal activation of the sensory elements of the nervous system was assessed by nuclear c-Fos expression in the dorsal horn of the spinal cord as well as the nucleus of the solitary tract fifty minutes after the LPS challenge. It was found that inhibiting prostaglandin synthesis prevented neuronal activation, this caused an increase in the circulatory levels of the early cytokine, tumor necrosis factor as well as changed glycemic and heart rate response. Pharmacological evidence points to regulation of these parameters via beta adrenergic receptors, nevertheless, this does not assess the role of different catecholamines capable of stimulating these receptors. The involvement of epinephrine in this antiinflammatory and glycemic reflex is approached by surgical removal of adrenal medullas, which is the main source of epinephrine. In conclusion the early activation of the sensory elements of the nervous system

is due to sensing of prostaglandin in the periphery while epinephrine is involved in regulation of early physiological reflex-like changes caused by endotoxin administration.

Disclosures: E. Santacruz-Martínez: None. **E. Soto-Tinoco:** None. **R. Buijs:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.08

Topic: F.05. Neuroimmunology

Title: T-cell activation in patients with multiple sclerosis on B-cell depleting therapies is as effective as that of general population in response to SARS-CoV-2 vaccination

Authors: *V. KIRSCHNER, G. FEUER, M. MALIN, J. LEI, J. LIU, M. ROCHE, J. LIN, R. ALFONSO, S. SADIQ;
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Abstract: Many patients with multiple sclerosis (MS) undergo immunosuppressive B-cell depleting therapies which can severely limit their humoral immunity against SARS-CoV-2 (CoV2) infection or response to vaccination. While robust antibody production is seen in immunocompetent individuals, recent studies show that B-cell depleting therapies inhibit efficient production of antibodies against CoV2 proteins due to reduction in circulating B-cells. It is unknown how these disease modifying therapies affect T-cell responses after CoV2 vaccination, and whether there is a correlation between CoV2 antibody levels and T-cell immunity.
Our ongoing single-center study aims to determine CoV2 spike protein-T-cell reactivity in fully vaccinated, B-cell depleted MS patients treated with rituximab or ocrelizumab, two well-characterized anti-CD20 monoclonal antibody drugs. We collected peripheral blood mononuclear cells (PBMCs) and serum samples from treated MS patients and healthy control individuals at 15-93 days after completing vaccination. CoV2 IgG response was determined using anti-spike protein-based serology and levels of proinflammatory cytokine (IL-2 and INF-Ɣ) release after protein-specific T-cell stimulation were measured *ex vivo* using an ELISpot assay.
We observe that from 23 vaccinated MS anti-CD20 treated patients studied, 19 (82.6%) showed defective humoral immunity. Interestingly, most CoV2 spike IgG negative patients (17 out of 19, 89.5%) presented a positive T cell response with strong IL-2 and INF-Ɣ expression. These results suggest the generation of a partial adaptive immune response to CoV2 vaccination in B-cell depleted individuals, driven by a functionally competent T-cell arm. Investigation into the role of T-cell response in these individuals is crucial to identifying their levels of protection against COVID-19.

Disclosures: V. Kirschner: None. **G. Feuer:** None. **M. Malin:** None. **J. Lei:** None. **J. Liu:** None. **M. Roche:** None. **J. Lin:** None. **R. Alfonso:** None. **S. Sadiq:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.09

Topic: F.05. Neuroimmunology

Support: Cure Alzheimer's Fund
NIMH Conte Grant

Title: In vivo two-photon imaging of meningeal B cells

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Abstract: The central nervous system (CNS) is ensheathed by meninges, protective membranes that harbor a rich diversity of immune cells. Among the adaptive immune cells that localize to the meninges, B cell populations have recently been observed within the dura proximal to calvarial bone marrow. Notably, a distinct wave of B cells colonizes the meninges during the early postnatal period, yet real-time characterization of the dynamics of these meningeal cells has remained elusive. We developed an *in vivo* approach to studying the dynamic interplay between B cells residing in the calvarial bone marrow and the meninges via two-photon imaging through the intact skull. Using custom headplates and fixation devices in juvenile mice aged P12-P17 expressing GFP under the control of Mb1 (*Mb1-Cre;mT/mG*), a B cell antigen receptor gene, we gained optical access to the bone marrow and dural sinuses. Transcranial imaging along the sagittal sinus confirmed both the presence of B cells in the dura and of specialized channels connecting the bone marrow and meninges for bidirectional B cell transport. B cells were relatively stable within both niches, with little movement and rare exchange of cells between compartments. However, administration of niche-disruptive drugs decreased the stability of B cells within their respective niches and increased trans-compartmental movement. Together, these results show that two-photon microscopy through the intact skull of transgenic mouse pups is a robust model in which to study the real-time dynamics of meningeal lymphocytes and the niche they reside in.

Disclosures: H.J. Barr: None. **A.J. Walker:** None. **T.M. Fisher:** None. **L. Dissing-Olesen:** None. **B.A. Stevens:** None.

Poster

P617. Regulating Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P617.10

Topic: F.05. Neuroimmunology

Support: CAPES/Print 88887.374124/2019-00
CRC Grant 950-231859
CIHR Grant 407016
CFI Grant 37439
NSERCC Grant RGPIN-2019-06824
BCF Grant 4758

Title: Nociceptor neurons control skin graft rejection

Authors: *T. EICHWALD[1], J.-C. WANG[1], M. AHMADI[1], M. BALOOD[1], K. ROVERSI[1], A. LATINI[2], S. TALBOT[1];
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Abstract: Nociceptor neurons control skin graft rejection
Tuany Eichwald [1,2], Jo-Chiao Wang [1], Maryam Ahmadi [1], Mohammad Balood [1], Katiane Roversi [1], Alexandra Latini [2], Sebastien Talbot [1]
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Skin grafting is an effective tool for treating various conditions such as third-degree burn, but the longevity of the transplant needs to be improved. Upon allogeneic graft, the host mounts an immune response against the transplant which results in its rejection. An effect managed clinically using immunosuppressive treatment. Our work in neuro-immunology has shown that skin nociceptor neurons control immune exhaustion against innate and adaptive immune cells. Here, we sought to test whether these neurons are activated in the context of skin grafting and, if so, do they promote immune-mediated rejection. To induce rejection, 8-week-old male C57BL6 (autograft) or BALB/c (allograft) mice underwent a 10mm skin biopsy which was immediately grafted to C57BL6 mice whose sensory neurons are either intact ($TRPV1^{wt}DTA^{fl/wt}$, $Na_V1.8^{wt}DTA^{fl/wt}$) or genetically ablated ($TRPV1^{cre}DTA^{fl/wt}$, $Na_V1.8^{cre}DTA^{fl/wt}$). To measure immune influx to the skin, the mice were also implanted with polyvinyl alcohol (PVA) sponges which were removed and immunophenotyped on day 1 and 3. In littermate control mice, we found that skin rejection occurs on day 19 in mice who received an autograft but on day 15 for the one that received an allograft. Interestingly, we discovered that sensory neuron ablated mice were protected from rejection, eliminating the allograft transplant on day 18. Next, we postulate

that the delay in rejection may be due to a reduced immune cells activation in nociceptor ablated mice or an increase in immune cells exhaustion. When compared with autograft, our data shows that allograft littermate control animals have higher immune infiltration, characterized by innate immunocytes on day one and adaptive immune cells on day 3. Interestingly, nociceptor depleted mice showed reduced number and activation (defined as PD1+) of skin infiltrating CD8+ and NK cells than allograft littermate control mice. Multiplex cytokine array also showed reduced skin IL-4 and MCP-1 levels in allograft sensory neuron ablated mice. Overall, sensory neuron ablation reduces immune influx and transplant rejection. These data support the existence of a maladaptive neuro-immune network mediating skin graft rejection and paved the way for sensory neuron targeted treatment to promote graft longevity.

Disclosures: T. Eichwald: None. **J. Wang:** None. **M. Ahmadi:** None. **M. Balood:** None. **K. Roversi:** None. **A. Latini:** None. **S. Talbot:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.01

Topic: G.05. Mood Disorders

Support: NIH Grant MH116900
NIH Grant HD097088
NIH Grant DA042078

Title: Broad histone serotonylation domains associate with neurodevelopmental gene expression programs

Authors: *J. C. CHAN[1], A. RAMAKRISHNAN[1], M. BEDFORD[2], L. SHEN[1], H. LI[3], I. S. MAZE[1];
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Abstract: Brain development is a complex organizational process that can be altered by genetic variants, environmental perturbations, aberrant monoaminergic signaling, and more. In particular, dysregulation of the serotonin (5-HT) system has been implicated in the pathophysiology of neurodevelopmental disorders including autism spectrum disorders (ASD), yet the molecular mechanisms underlying 5-HT contribution to neurodevelopment remains unclear. Recently, our lab identified a novel epigenetic role for 5-HT, whereby this biogenic monoamine can be transamidated to glutamine 5 of histone H3 (H3Q5). Deposition of 5-HT at this site stabilizes neighboring H3K4me3, resulting in the combinatorial H3K4me3Q5ser modification that recruits specific regulatory machinery to increase permissive transcription.

Interestingly, an additional role of H3K4me3 ‘broad’ domains has been described. Broad H3K4me3 peaks that span over 4 kb correspond to enhanced transcriptional activation and associate with cell-specific processes, such as synaptic signaling in neurons. In postmortem brains from ASD patients, aberrant spreading of H3K4me3 domains has been observed, though how this dysregulation occurs is unclear. Our current studies examine histone serotonylation involvement in the organization of H3K4me3 spreading to impact neurodevelopment. To determine whether H3Q5 serotonylation associates with broad H3K4me3 peaks, we performed chromatin immunoprecipitation coupled to sequencing of H3K4me3Q5ser in mouse embryonic forebrain tissues. Indeed, characterization of H3K4me3Q5ser peaks indicate this dual modification exhibits broad patterning, occupying 4-to-35 kb across the genome. Moreover, broad H3K4me3Q5ser domains associate with loci that enrich for neurodevelopment-associated pathways. Spreading of H3K4me3 peaks is regulated, in part, by the enzyme Lysine methyltransferase 2e (Kmt2e). To determine whether H3Q5 serotonylation influences Kmt2e recruitment, we used a high-throughput methyl domain reader array that evaluates binding between recombinant chromatin reader domains and modified histone tail peptides. Along with isothermal calorimetry, we show that the Kmt2e PHD finger binds H3K4me3Q5ser peptides more extensively than H3K4me3 alone, suggesting H3Q5 serotonylation may enhance Kmt2e recruitment to regulate H3K4me3 spreading. Ongoing work focuses on the contribution of Kmt2e to organization of H3K4me3Q5ser broad domains and subsequent impact on neuronal gene expression programs. These studies offer a novel role for histone serotonylation that, if disrupted, may contribute to the etiology of neurodevelopmental disorders.

Disclosures: J.C. Chan: None. **A. Ramakrishnan:** None. **M. Bedford:** None. **L. Shen:** None. **H. Li:** None. **I.S. Maze:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.02

Topic: G.05. Mood Disorders

Support: DP1 DA042078
R01 MH116900
R01 HD097088

Title: Identification and functional characterization of CaMKII glutamine 285 dopaminylation in brain

Authors: *A. F. STEWART[1], R. M. BASTLE[1], S. MITRA[2], R. THOMPSON[3], S. L. FULTON[1], A. LEPACK[1], L. FARRELLY[1], B. CETIN[1], Y. LU[1], H. MOLINA[4], T. W. MUIR[3], D. M. DIETZ[2], I. S. MAZE[1];

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Abstract: Monoamines are traditionally regarded as chemical signals that act extracellularly on surface bound receptors in brain. However, recent work from our lab demonstrates that non-vesicular monoamines have receptor-independent functions that are mediated via transamidation reactions onto glutamine residues of protein substrates. We selectively isolated dopaminylated proteins in brain utilizing a novel endogenous chemical tagging method to specifically immunoprecipitate dopaminylated proteins. Immunoprecipitations coupled to mass spectrometry identified novel dopaminylated proteins in synaptic fractions isolated from nucleus accumbens (NAc), including multiple isoforms of CaMKII, with their dopaminylation states regulated by drugs of abuse (e.g., heroin self-administration/SA). We further characterized CaMKII dopaminylation utilizing *in vitro* enzymatic assays coupled to mass spectroscopy, where we identified glutamine 285 (Q285) as the selective site of dopaminylation on γCaMKII. γCaMKII is a synapse-to-nucleus signaling molecule involved in the shuttling of calmodulin (CaM) to the nucleus to promote phosphorylation CREB, among other downstream signaling events. Utilizing primary striatal-cortical co-cultures, we identified a blockade of CaM shuttling upon dopamine treatment, despite γCaMKII's entry to the nucleus. We additionally observed that this phenomenon is dopamine receptor independent and provide evidence of a direct role for Q285dop on CaM binding. Ultimately, we hypothesize that the presence of this modification on γCaMKII may change our conceptualizations of post-synaptic dopaminergic signaling in brain, where dopamine may not only act at the cell surface, but also infiltrates post-synaptic cells and directly modifies the proteins therein to mediate important cellular signaling events.

Disclosures: A.F. Stewart: None. **R.M. Bastle:** None. **S. Mitra:** None. **R. Thompson:** None. **S.L. Fulton:** None. **A. Lepack:** None. **L. Farrelly:** None. **B. Cetin:** None. **Y. Lu:** None. **H. Molina:** None. **T.W. Muir:** None. **D.M. Dietz:** None. **I.S. Maze:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.03

Topic: G.05. Mood Disorders

Support: MQ15FIP100011, MQ: Transforming Mental Health Research Fellowship

Title: Histone serotonylation: a novel regulator of stress-induced neuroplasticity

Authors: *A. AL-KACHAK[1], L. FARRELLY[1], S. FULTON[1], R. BASTLE[1], A. LEPACK[1], P. SAFOVICH[1], Y. LU[1], E. NEWMAN[2], H. COVINGTON[2], K. MICZEK[2], C. MENARD[3], A.

RAMAKRISHNAN[1], A. AGUSTINUS[4], S. RUSSO[1], L. SHEN[1], Y. DAVID[4], I. MAZE[1]; [1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Dept. of Psychology, Tufts Univ., Medford, MA; [3]Dept. of Psychiatry and Neuroscience, Fac. of Med. and CERVO Brain Res. Ctr., Univ. Laval, Ville de Québec, QC, Canada; [4]Chem. Biol. Program, Mem. Sloan-Kettering Cancer Ctr., New York, NY

Abstract: Background: The field of neuroepigenetics has recently implicated chromatin phenomena in the etiology of major depressive disorder (MDD). While it has been demonstrated that dysregulation of histone posttranslational modifications may be involved in the deleterious transcriptional processes that promote physiological maladaptations in MDD, the field still has only a limited understanding of the underlying mechanisms contributing to this disorder. Data from our laboratory suggest potential alternative mechanisms of action for monoamines, where the presence of serotonin in the dorsal raphe nucleus (DRN) may directly mediate transcriptional responses related to various forms of serotonergic plasticity, and the subsequent mediation of mood.Methods: Mice were subjected to chronic social defeat stress (CSDS) and behavioral response was analyzed via social interaction (SI), classified as either stress-susceptible or resilient. Each cohort was 1) sacrificed 24h post-CSDS, 2) subjected to 30 days of fluoxetine vs. water treatment, or 3) underwent viral surgery to block serotonylation. Human tissue were obtained from a brain bank.Results: We detected a decrease in serotonylation and an enrichment in ubiquitin pathways in MDD patients and male/female mice 48h post-CSDS. Serotonylation levels were significantly increased 30 days post-CSDS, an effect that is reversed with fluoexetine treatment. Blocking serotonylation resulted in significantly greater SI than control groups.Conclusion: Globally blocking serotonylation in DRN promotes a resilient response to CSDS, and there is an accumulation of serotonylation that promotes a long-term vulnerability to stress, possibly through ubiquitination.

Disclosures: A. Al-Kachak: None. **L. Farrelly:** None. **S. Fulton:** None. **R. Bastle:** None. **A. Lepack:** None. **P. Safovich:** None. **Y. Lu:** None. **E. Newman:** None. **H. Covington:** None. **K. Miczek:** None. **C. Menard:** None. **A. Ramakrishnan:** None. **A. Agustinus:** None. **S. Russo:** None. **L. Shen:** None. **Y. David:** None. **I. Maze:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.04

Topic: G.05. Mood Disorders

Support: 1F31MH124425-01
P50 MH096890
R01MH109677

U01MH116442
R01MH110921

Title: Astrocytic ZBTB7A promotes aberrant cortical plasticity associated with Major Depressive Disorder

Authors: *S. FULTON[1], J. BENDL[2], J. FULLARD[3], A. AL-KACHAK[3], A. LEPACK[3], R. M. BASTLE[5], A. F. STEWART[4], S. J. RUSSO[6], K. GLEASON[8], C. A. TAMMINGA[8], A. FAKIRA[7], R. D. BLITZER[7], P. ROUSSOS[2], I. S. MAZE[2];
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Abstract: The overall risk for developing major depressive disorder (MDD) is determined by a complex interaction between heritable genetic variants that confer increased vulnerability to MDD in the presence of stress exposure and other adverse experience. Mechanistic understanding of this gene-environment interface requires characterization of epigenomic processes within the unique regulatory context of disease-relevant cell types, but to date this approach has not been applied to MDD. Here, we use cell population-specific chromatin accessibility profiling to map the active repertoire of gene regulatory elements in a brain region relevant to MDD, the orbitofrontal cortex (OFC). Although relatively understudied in human MDD, the orbitofrontal cortex (OFC) integrates internal homeostatic states, emotion, and motivating stimuli in order to flexibly guide behavior. Functional imaging studies consistently find significant OFC dysfunction in MDD patients, however, the molecular and cellular substrates underlying these neuroplastic changes in the OFC are not well understood. Here, we utilized FANS coupled - Assay for Transposase-Accessible Chromatin-Sequencing (ATAC-seq) to capture the chromatin regulatory signature of both neuronal and glial cells in the OFC of MDD vs. control patients. We mapped genetic risk for MDD to active chromatin regions in non-neuronal OFC cell-types, and identified MDD-specific open chromatin regions (OCRs), present exclusively in non-neuronal OFC cells. Characterization of these MDD-specific OCRs revealed a key role for the chromatin remodeling protein ZBTB7A, which is known to facilitate transcription of NF-kB inflammation pathway genes, and is enriched in astrocyte cell-types, though its role in neuropsychiatric disease has not been explored. In a series of functional validation studies, we utilized a preclinical rodent model of stress vulnerability to show that overexpressing (OE) ZBTB7A specifically in rodent OFC astrocytes is sufficient to induce behavioral deficits, widespread inflammatory gene expression, and aberrant neuronal hyperactivity in response to a mild stressor. Importantly, our data demonstrate that ZBTB7A OE in OFC astrocytes reverses the same changes in gene expression and neuronal activity that support adaptive behavioral responses to mild stress, suggesting a key role for astrocyte plasticity in vulnerability to stress exposure. Together, these data demonstrate that epigenetic regulation of inflammatory signaling in astrocytes impairs neuroadaptive responses to stress, with direct implications for the role of astrocyte plasticity in OFC dysfunction and MDD pathology.

Disclosures: S. Fulton: None. **J. Bendl:** None. **J. Fullard:** None. **A. Al-Kachak:** None. **A. Lepack:** None. **R.M. Bastle:** None. **A.F. Stewart:** None. **S.J. Russo:** None. **K. Gleason:** None. **C.A. Tamminga:** None. **A. Fakira:** None. **R.D. Blitzer:** None. **P. Roussos:** None. **I.S. Maze:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.05

Topic: G.05. Mood Disorders

Support: MH111276

Title: Seasonal expression of 5-alpha reductase in corticolimbic brain regions of a diurnal rodent

Authors: *A. COSTELLO[1], K. LINNING-DUFFY[1], C. VANDENBROOK[1], J. S. LONSTEIN[2], M. BORTOLATO[3], L. YAN[1];
[1]Psychology, [2]Neurosci Program, Michigan State Univ., East Lansing, MI; [3]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT

Abstract: 5-Alpha-Reductase (5AR) is a key enzyme catalyzing the biosynthesis of numerous neurosteroids that regulate affect and cognition. In humans, administration of 5AR inhibitors is associated with increased risk for depression. In laboratory mice and rats, higher levels of 5AR in limbic structures are associated with reduced anxiety- and depressive-like behaviors, while downregulating 5AR leads to increased anxiety- and depression-like behaviors. In songbirds, brain 5AR expression has been reported to undergo seasonal changes. However, it is unclear whether the same fluctuations occur in mammals, and the extent to which the changes in 5AR are involved in Seasonal Affective Disorder (SAD), a major depressive disorder caused by seasonal fluctuation in the level of ambient light. To begin exploring these questions, the present study utilized a diurnal rodent, the Nile grass rat (*Arvicanthis niloticus*). Unlike the most commonly used laboratory rodents (i.e., mice or rats) that are nocturnal and sleep most during the day, Nile grass rats sleep most at night, like humans. Our group's previous studies using grass rats have found that reducing daytime light exposure, which is etiologically relevant to SAD in humans, leads to increased depression- and anxiety-like behaviors, impaired spatial memory, and disrupted male copulatory behaviors. To investigate the seasonal regulation of 5AR, male and female grass rats housed in 12:12 hr light/dark (LD) cycle were randomly assigned to two conditions involving different light intensity during the day, a summer-like bright light (1000 lux) and winter-like dim light (50 lux) for 4 weeks. Gene expression of two 5AR isoforms Srd5a1 and Srd5a2 within the prefrontal cortex (PFC), anterior cingulate cortex (ACC), dorsal hippocampal subregion CA1, and the basolateral amygdala (BLA) were quantified using real-time qRCR. The results revealed that winter- or summer-like daylight intensity

condition had no significant effect on the expression of Srd5a1 in the brain regions examined. However, there were numerous sex differences found. Within BLA, males had higher expression of srd5a1 than females and there was a sex by light interaction for Srd5a2. Within the PFC, males had higher expression of Srd5a2 than females. The sex- and region-specific responses of 5AR genes to light condition indicate complex and dynamic changes in the biosynthesis of neurosteroids over season in the diurnal brain and suggest the involvement of 5AR in the behavioral detriments caused by daytime light deficiency.

Disclosures: A. Costello: None. **K. Linning-Duffy:** None. **C. Vandenbrook:** None. **J.S. Lonstein:** None. **M. Bortolato:** None. **L. Yan:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.06

Topic: G.05. Mood Disorders

Support: Ford Foundation Postdoctoral Fellowship to MRC
NIMH R01-057440 to AAG

Title: Postpartum scarcity-adversity drives aberrant maternal behavior and induces a hypodopaminergic start in the rat dam and adult female offspring

Authors: *M. RINCÓN-CORTÉS, A. GRACE;
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Abstract: Postpartum adversity is one of the strongest predictors for the emergence of postpartum depression (PPD) in humans and a translational risk factor widely employed in relevant rodent models. Parental care is disturbed under conditions of adversity and/or chronic stress, including low resource environments, and in PPD. Nonetheless, the neural changes associated with these adversity-induced maladaptive behavioral states remain poorly understood. Here we show that postpartum scarcity-adversity can be modeled in rats by providing the rat dam with limited bedding and nesting (LBN) materials, which stresses the dam and mimics the effects of a stressful and low resource environment in potentiating maltreatment/neglect in humans. Indeed, LBN exposure from postpartum days (PD) 2-9 increased adverse caregiving behaviors directed at pups, impaired pup retrieval and increased FST immobility (t-tests: n=7-9 per group, $p < 0.05$). Given that mesolimbic dopamine (DA) function is an important mechanism for motivated maternal behavior and implicated in the pathophysiology of PPD, we assessed the impact of postpartum scarcity-adversity on *in vivo* electrophysiological properties of ventral tegmental area (VTA) DA neurons at two timepoints (n=5-7 per group). We found reduced numbers of active VTA DA neurons in LBN dams at PD 9-10 ($p < 0.01$) but not PD-21 ($p=0.54$),

suggesting a transient impact on VTA population activity in LBN dams. Finally, we assessed the transgenerational impact of early life scarcity-adversity exposure on VTA DA function by conducting VTA recordings in adult female offspring and found a long-lasting attenuation in VTA DA activity (n=6-8 per group, $p < 0.01$). These findings highlight a link between adversity-induced deficits in DA function and disrupted maternal behavior, suggesting the VTA/mesolimbic DA system as a potential mechanism by which postpartum scarcity-adversity drives disrupted maternal behavior, and early postnatal programming of adult VTA function in the offspring.

Disclosures: M. Rincón-Cortés: None. **A. Grace:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.07

Topic: G.05. Mood Disorders

Support: Swedish Research Council

Title: Cortico-cortical relay of acoustic information to mouse Orbitofrontal cortex: relevance for neuropsychiatric disorders.

Authors: *A. TRIPATHI, S. SULIS SATO, P. MEDINI;
Integrative Medial Biol. Dept., Umeå Univ., Umea, Sweden

Abstract: Cortico-cortical relay of acoustic information to mouse orbifrontal cortex: relevance for neuropsychiatric disorders.
Anushree Tripathi, Sebastian Sulis Sato and Paolo Medini
The Orbitofrontal cortex (OFC) is salient for emotional regulation due to its reciprocal interactions with central brain areas such as the amygdala. These affective responses, disturbed in several psychiatric disorders are often driven by acoustic inputs. Indeed, auditory hallucinations are common hallmark of psychosis in both mood and thought disorders. Here we investigated the underlying cortico-cortical connectivity relaying acoustic information to mouse OFC. We also explored the sensitivity of this pathway to neuromodulators such as dopamine and neurosteroids as well as to sensory gating, impaired in dysexecutive syndromes. Retrograde tracers in the OFC revealed input cells in all auditory cortices, and clear acoustic responses were measured in OFC by local field potentials. Auditory responses were abolished by pharmacological and chemogenetic inactivation of the above identified pathway. The OFC auditory responses were reduced by local application of both dopaminergic agonist, Apomorphine and neurosteroid Allopregnanolone. Noticeably, apomorphine action lasted more in OFC compared to auditory areas and was modality-specific, whereas neurosteroids´ action

was sex-specific. Finally, the OFC acoustic responses reverberated to auditory cortex via feedback connections and displayed sensory gating, a phenomenon of local origin, not detectable in input auditory cortices.Taken together, our data demonstrates the cortico-cortical connectivity behind acoustic responses in the OFC, their dramatic sensitivity to dopamine and neurosteroid modulation, and to sensory gating by local OFC microcircuit action. Further illustration of the role of the OFC input-output circuits in auditory hallucinations can be studied in mice with targeted and clinically relevant mutations, in terms of behavioral responses to emotionally salient sounds.

Disclosures: A. Tripathi: None. **S. Sulis Sato:** None. **P. Medini:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.08

Topic: G.05. Mood Disorders

Support: NIH R01 MH108562
NIH R01 DA040630
NIH T32 MH064913

Title: Sex-specific effects of photoperiod on dopamine signaling in the nucleus accumbens

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Abstract: Circadian photoperiod, or day length, has a powerful influence on the function of neural circuits responsible for behavior. Photoperiodism is the response of an organism to seasonal changes in day length. Seasonal Affective Disorder (SAD) is an example of photoperiodism in which depressive symptoms are more prevalent and severe during winter months. Previously, our lab developed a novel mouse model that demonstrates photoperiodic input shapes long-term function of dorsal raphe (DR) serotonin neurons as well as affective behavior. However, little is known about an extended circuit beyond the DR that is sensitive to photoperiod and ultimately programs behavior. The DR projects to reward circuit regions that we hypothesize are also impacted by photoperiod. Within the reward circuit, the nucleus accumbens (NAc) is a ventral striatal region that integrates excitatory and neuromodulatory inputs to coordinate behavior. The NAc is highly implicated in reward processing and affective behaviors-thus, we predict that photoperiod-driven changes to the NAc result in changes to behavior. Here

we show that photoperiod programs dopamine (DA) dynamics in the NAc and DA-dependent behavior. Male and female mice were exposed to short (8hrs light, 16hrs dark), long (16hrs light, 8hrs dark), or equinox (12hrs light, 12hrs dark) photoperiod conditions. Using *ex-vivo* fast-scan cyclic voltammetry, we found that female mice in the long, summer-like photoperiod condition showed elevated DA release and reuptake rate in the NAc. Interestingly, male mice did not show differences in DA measures by photoperiod. We also assessed cocaine-induced hyperlocomotion in male and female short, long, and equinox mice. Short photoperiod female mice show a blunted locomotor response to cocaine, while male mice showed no differences by photoperiod. Our results indicate that photoperiod has a sex-specific effect on DA dynamics in the NAc that ultimately affects behavior. These results further our understanding of sex differences in mood disorders, especially in SAD. More importantly, we demonstrate that sex and environmental factors play a large role in DA dynamics and DA-dependent behavior, and both lighting conditions and the inclusion of females should be taken into consideration in future research.

Disclosures: A.N. Jameson: None. **J.K. Siemann:** None. **J.R. Melchior:** None. **E.S. Calipari:** None. **D.G. Winder:** None. **C.A. Grueter:** None. **B.A. Grueter:** None. **D.G. McMahon:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.09

Topic: G.05. Mood Disorders

Support: NIH Grant MH106460
NIH Grant MH115241
NIH Grant MH111601
NARSAD Young Investigator Grant

Title: The suprachiasmatic nucleus regulates anxiety-like behavior in mice

Authors: *K. A. PETERSEN[1], C. A. VADNIE[2], L. EBERHARDT[1], M. HILDEBRAND[1], A. J. CERWENSKY[1], H. ZHANG[1], J. N. BURNS[1], D. BECKER-KRAIL[1], L. DEPOY[1], R. LOGAN[3], C. A. MCCLUNG[1];
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Abstract: Individuals suffering from mood and anxiety disorders often show significant disturbances in sleep and circadian rhythms. Animal studies indicate that circadian rhythm disruption can cause increased depressive and anxiety-like behavior, but the underlying mechanisms are unclear. One potential mechanism to explain how circadian rhythms are contributing to mood disorders is through dysregulation of the suprachiasmatic nucleus (SCN) of

the hypothalamus, known as the “master pacemaker”. To investigate the role of the SCN in regulating depressive and anxiety-like behavior in mice, we directly manipulated the neural activity of the SCN using three optogenetic stimulation paradigms. First, acute stimulation of the SCN in the active phase (circadian time 14-16, CT14-16) during behavioral testing resulted in increased anxiety-like behavior. Chronic stimulation of the SCN late in the active phase (CT21) to advance rhythms shortened the period and dampened the amplitude of homecage activity rhythms. Lastly, repeated stimulation of the SCN at unpredictable times during the active phase dampened, fragmented, and reduced the stability of homecage activity rhythms. In both chronic SCN optogenetic stimulation paradigms, dampened homecage activity rhythms (decreased amplitude) were associated with increased measures of anxiety-like behavior. Unexpectedly the change in period of homecage activity rhythms was not directly associated with any mood-related behavior. Furthermore, we did not observe consistent correlations between homecage activity amplitude and depressive-like behavior in stimulated mice. Taken together, these results indicate that SCN-mediated dampening of rhythms is directly correlated with increased anxiety-like, but not depressive-like behavior in mice. This work is an important step in understanding how specific SCN neural activity disruptions affect mood and anxiety-related behavior.

Disclosures: K.A. Petersen: None. **C.A. Vadnie:** None. **L. Eberhardt:** None. **M. Hildebrand:** None. **A.J. Cerwensky:** None. **H. Zhang:** None. **J.N. Burns:** None. **D. Becker-Krail:** None. **L. Depoy:** None. **R. Logan:** None. **C.A. McClung:** None.

Poster

P706. Brain Mechanisms Underlying Neuropsychiatric Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P706.10

Topic: G.05. Mood Disorders

Support: AMERICAN HEART ASSOCIATION 19PRE34370044 (AGK)
R01MH123093-01
Scholars in BioMedical Sciences Program (AGK)

Title: Disruption of the homeostasis and progression of oligodendroglial lineage, associating immune oligodendrocytes with prefrontal cortex hypomyelination in a depression mouse model

Authors: *A. G. KOKKOSIS, M. M. MADEIRA, S. E. TSIRKA;
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Abstract: Background Major Depressive Disorder (MDD; depression) is a chronic debilitating illness affecting yearly 350 million people worldwide. Emerging evidence shows that oligodendrogenesis and myelination are highly sensitive to stressful experiences and psychosocial input, with significant oligodendrocyte progenitor cell (OPC) reductions and white

matter deficits being reported in MDD patients. In a mouse model of depression, we demonstrate that chronic psychosocial stress can induce significant compromises of oligodendroglial-lineage (OLN) homeostasis, associating immune oligodendrocytes (ImOL) with OPC maturation arrest and myelin deficits. **Methods** The Repeated Social Defeat Stress (RSDS) paradigm (10 days) was utilized to study the early (6 days) and post RSDS stages (10+5 days and 10+15 days) in 8-12-week-old male C57BL/6J, *CX3CR1*-GFP$^+$, *CSPG4*-EGFP$^+$ mice. Behavioral tasks were performed to categorize the socially defeated mice as susceptible (S; depressive-like) and resilient (R; non-depressive) to stress. Naïve non-stressed (C) mice were included in the study. The study focused on the MDD-affected areas: prefrontal cortex (mPFC), ventral and lateral orbital cortex (VO-LO) and forceps minor (fmi). **Results** A time-dependent characterization of OLN cells revealed significant long-lasting losses of OPC populations (PDGFRα$^+$, CSPG4$^+$), and severe myelin deficits (MBP$^+$, CNP$^+$, MOG$^+$, MOBP$^+$) in the mPFC of the S mice. In addition, chronic stress led to a transient proliferation surge (BRDU$^+$, Ki67$^+$) of OPCs in early RSDS and a subsequent differentiation to immature (Pre-OLs; O4$^+$) and mature OLs (GST-π$^+$) post-RSDS. In addition, chronic psychosocial stress led to excessive oxidative stress (2-OH-Ethydine), activation of integrated stress responses (p-eIF2α/ATF4/CHOP axis), and impaired morphology in OPCs (Sholl and branched-structure analyses), leading to OPC apoptosis and OL reductions post-RSDS. Using a sn-RNAseq dataset from the PFC of MDD patients (Nagy et al., 2020), we were able to identify a novel OL population, the Im-OL, expressing immune-related components: MHCII, C3, P2RY12, in the S groups post-RSDS. Of importance, significant microglial recruitment (CX3CR1) and phagocytosis of MHCII-tagged myelin (CX3CR1$^+$CNP$^+$ MHCII$^+$) in S mice suggest that ImOL populations might act as recruiters of innate immunity and concomitant myelin deficits during depression. **Conclusions** Our data exhibit that chronic stress can significantly compromise the OLN homeostasis and lead to OPC/OL reductions, suggesting that the ImOLs might contribute as an instigator of myelin deficits in a depression mouse model.

Disclosures: A.G. Kokkosis: None. **M.M. Madeira:** None. **S.E. Tsirka:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.01

Topic: G.09. Drugs of Abuse and Addiction

Title: The effects of sex and ovarian hormones on morphine-induced anxiety and withdrawal behaviors

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Abstract: Addiction is a chronic and relapsing brain disease that levies a high cost on both the individual and on society. Although women have been traditionally understudied in addiction, there is a consensus that female and male populations exhibit unique profiles of drug use and addiction for multiple drugs of abuse (including opioids). For example, women (and female rats) exhibit a “telescoping effect” in which they transition from first use to addiction more quickly than men (and male rats). Differences in opiate withdrawal are also present, with male rats displaying more severe symptoms of somatic withdrawal earlier on, and female rats displaying a more protracted withdrawal syndrome. Ovarian hormones, such as estradiol and progesterone, have also been implicated in addiction. For example, high circulating levels of estradiol are associated with increased drug-seeking behavior. High circulating progesterone, on the other hand, is associated with decreases in drug-seeking behavior. Much less, however, is known about how ovarian hormones might influence somatic withdrawal and anxiety-related behaviors in response to opiate withdrawal. To understand this relationship, we performed a study in which female and male rats received experimenter-administered escalating doses of morphine (or saline) twice daily for ten days, followed by somatic withdrawal observation (12, 24, 36, 48, 60, 72, 84, 96, and 108 hrs into withdrawal), elevated plus maze testing (12 and 108 hrs into withdrawal), and light-dark box testing (12 and 108 hrs into withdrawal). Prior to the initiation of the study, all female rats received ovariectomies and all male rats received sham surgeries. Following surgery, a four day cycle of subcutaneous hormone supplementation was provided to mimic cycling in the female rats (First Day - 0.1ml 5µg estradiol; Second Day - 0.1ml 5µg estradiol; Third Day - 0.1ml 250µg progesterone; Fourth Day - peanut oil). Male rats also received daily injections of peanut oil, but never received estradiol or progesterone. To understand the extent to which ovarian hormones contribute to anxiety during earlier and later withdrawal, multiple groups of female rats received staggered hormone supplementation so that estradiol or progesterone levels were highest during anxiety testing. Very preliminary evidence suggests that regardless of hormone treatment, morphine-treated female rats display more anxiety-related behavior compared to their matched saline-treated groups both 12 and 108 hours into withdrawal (a phenomenon not yet observed in male rats). The running of additional planned cohorts as well as the analysis of somatic withdrawal observation is currently underway.

Disclosures: T. Dennis: None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.02

Topic: G.09. Drugs of Abuse and Addiction

Support: The project described was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, Award Number UL1TR002544.

Title: Intranasal administration of GDNF plasmid DNA nanoparticles reduces reinstatement of intravenous drug self-administration in a rat model of opioid use disorder (OUD)

Authors: *B. L. WASZCZAK[1], F. M. VASSOLER[2], K. E. BUDGE[3], S. B. ISGATE[3], H. MOJAHED YAZDI[4], U. K. VANI[4], E. N. POTHOS[4], O. SESENOGLU-LAIRD[5], L. PADEGIMAS[5], R. C. MOEN[5], M. J. COOPER[5], E. M. BYRNES[3];
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Abstract: Relapse is a persistent threat to long term recovery for individuals with OUD. During recovery, craving is a key factor in the decision to reinitiate drug use. Intravenous self-administration reinstatement models provide a measure of relapse-related drug seeking behavior with high construct validity and provide a measure of "wanting" or "craving". Glial cell-line derived neurotrophic factor (GDNF) has been proposed as a therapy to reduce relapse in substance use disorders. Here, we assessed the effects of intranasal delivery of GDNF plasmid DNA nanoparticles (NPs) on relapse vulnerability by examining context-induced (spontaneous) reinstatement in long access (6h/day) oxycodone (OXY) self-administering rats following 30 days of forced abstinence. Adult male Sprague Dawley rats were implanted with jugular catheters and trained to selectively press an active lever for OXY (0.1 mg/kg/infusion; 6 h/day; 12 training days) using a fixed ratio 1 (FR1) schedule for 9 days followed by FR5 for 3 days to increase responding. On Day 13 all animals were tested for their level of motivated responding for OXY using a progressive ratio (PR) schedule. The PR schedule increases response requirements (number of lever presses) after each drug infusion, which tests animals' motivation to obtain the drug by increasing the effort required to obtain a single dose. On Day 14 animals were anesthetized with ketamine/dexmedetomidine and intranasally administered either saline vehicle or pGDNF DNA NPs (90 μg DNA) with groups counterbalanced based on both their total drug intake (comparable OXY exposure) and their responding during PR (comparable willingness to work for OXY). All animals then underwent forced abstinence for 30 days. Context-induced reinstatement was tested by returning all rats to the self-administration chamber and measuring active lever pressing over 90 min. Only saline was available in the syringe. Active lever pressing served as a measure of drug seeking. Results indicated that intranasal pGDNF NPs significantly decreased reinstatement in rats that exhibited similar amounts of OXY intake and progressive ratio (PR) responding prior to drug infusion ($p=0.028$; OXY+veh n=12, OXY+ pGDNF NPs n=12). Even relatively high responders treated with pGDNF NPs demonstrated reduced reinstatement behavior, suggesting efficacy over a range of intake levels and in animals demonstrating robust PR responding. These data support our hypothesis that intranasal GDNF DNA NPs may correct a prolonged reward deficit instigated by the chronic use of opioids, and they strongly endorse the clinical translational potential of intranasal pGDNF DNA NPs as a treatment to reduce opioid relapse.

Disclosures: B.L. Waszczak: None. **F.M. Vassoler:** None. **K.E. Budge:** None. **S.B. Isgate:** None. **H. Mojahed Yazdi:** None. **U.K. Vani:** None. **E.N. Pothos:** None. **O. Sesenoglu-Laird:** None. **L. Padegimas:** None. **R.C. Moen:** None. **M.J. Cooper:** None. **E.M. Byrnes:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA K01DA050908 to ZJW
University of Kansas start up funding

Title: Early social isolation stress increases addiction vulnerability to heroin and alters c-Fos expression in mesocorticolimbic system

Authors: *Z. WANG, A. SINGH, Y. XIE;
Univ. of Kansas, Lawrence, KS

Abstract: ***Rationale***: Adverse psychosocial factors during early childhood or adolescence compromise neural structure and brain function, inducing susceptibility for many psychiatric disorders such as substance use disorder. Nevertheless, the mechanisms underlying early life stress-induced addiction vulnerability is still unclear, especially for opioids. ***Objectives***: To address this, we used a mouse heroin self-administration model to examine how chronic early social isolation (ESI) stress (5 weeks, beginning at weaning) affects the behavioral and neural responses to heroin during adulthood. ***Results:*** We found that ESI stress did not alter the acquisition for sucrose or heroin self-administration, nor change the motivation for sucrose on a progressive ratio schedule. However, ESI stress induced an upward shift of heroin dose response curve in female mice, and increased motivation and seeking for heroin in both sexes. Furthermore, we examined the neuronal activity (measured by c-Fos expression) within the key brain regions of the mesocorticolimbic system, including prelimbic cortex (PrL), infralimbic cortex (IL), nucleus accumbens (NAc) core and shell, caudate putamen, and ventral tegmental area (VTA). We found that ESI stress dampened c-Fos expression in PrL, IL and VTA after 14-day forced abstinence, while augmented the neuronal responses to heroin-predictive context and cue in IL and NAc core. Moreover, ESI stress disrupted the association between c-Fos expression and attempted infusions during heroin-seeking test in PrL. ***Conclusions***: These data indicate that ESI stress leads to increased seeking and motivation for heroin, and this may be associated with distinct changes in neuronal activities in different subregions of the mesocorticolimbic system

Disclosures: Z. Wang: None. **A. Singh:** None. **Y. Xie:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA025634 (MFR)

Title: Modulation of reward sensitivity by morphine administration and physiological state

Authors: *R. M. DONKA, B. G. RODRIGUEZ, T. M. HSU, M. F. ROITMAN, J. D. ROITMAN;
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Abstract: Physiological need states such as hunger and thirst drive organisms to perform goal-directed motivated behavior. Reward-circuitry in motivated behavior may be engaged by the positively reinforcing properties of needed food and fluid or by the negatively reinforcing properties of alleviation of the need state and its associated negative affect. In drug addiction, the induction of negative affect and subsequent alleviation through drug taking supports relapse through similar negative reinforcement. Here we examine changes in sensitivity of intracranial self-stimulation of the medial forebrain bundle under differing physiological need states as an index of reward sensitivity. We hypothesized that acute states of physiological need would increase reward sensitivity, while chronic states of physiological need would decrease reward sensitivity. Additionally, we hypothesized morphine withdrawal would decrease reward sensitivity. Stimulating electrodes were implanted in the medial forebrain bundle at the level of the lateral hypothalamus and animals were trained to lever press for stimulation (n = 17). Using a rate-frequency protocol we determined the threshold stimulation frequency (theta) that was reinforcing for each individual subject. We then measured how changes in physiological state altered theta and overall task engagement using a within-subjects design. We found that acute water deprivation, acute food deprivation, and chemogenetic activation of glutamatergic neurons in the subfornical organ (SFO) in water-sated animals decreased theta, indicating an increase in reward sensitivity compared to states of satiety. Additionally, total lever pressing increased in states of acute physiological need indicating a higher level of task engagement. We observed a small increase in theta values under chronic water restriction, indicating a decrease in reward sensitivity. Morphine administration and withdrawal both increased thetas, indicating a decrease in reward sensitivity. Lever pressing was significantly reduced during morphine administration and returned to baseline during withdrawal. Collectively, these results suggest that acute states of physiological need generally increase reward sensitivity while chronic need states generally reduce reward sensitivity despite specificity in food and fluid intake in response to need.

Disclosures: R.M. Donka: None. **B.G. Rodriguez:** None. **T.M. Hsu:** None. **M.F. Roitman:** None. **J.D. Roitman:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.05

Topic: G.09. Drugs of Abuse and Addiction

Support: R01-DA031900
Commonwealth Universal Research Enhancement Program (PA Dept. Health)

Title: Intermittent Access to Oxycodone Engenders Robust Drug-Seeking and Dopaminergic Changes in the Nucleus Accumbens

Authors: *K. R. SAMSON, R. A. ESPAÑA;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: One of the major barriers in successful treatment of opioid use disorder is the persistence of drug craving during periods of abstinence, which contribute to high relapse rates. Dopamine neurotransmission in the mesolimbic pathway has been implicated in opioid reward and reinforcement, but the precise mechanisms by which dopamine may drive drug craving has not been elucidated. To examine the extent to which opioid craving during abstinence is associated with alterations in dopamine neurotransmission, female and male rats self-administered oxycodone under an intermittent access schedule of reinforcement. Following self-administration, rats underwent a forced abstinence period. Cue-induced seeking tests were conducted on the first day of abstinence and at the end of the 14-day abstinence period to assess oxycodone craving. Eighteen hours following the final seeking test, we performed *ex vivo* fast scan cyclic voltammetry recordings in the nucleus accumbens core. Preliminary results suggest that intermittent access to oxycodone engendered distinct behavioral phenotypes, yielding a group of “low-seeking” rats and a group of “high-seeking” rats. Interestingly, both groups displayed dysfunctional dopamine transmission, compared to oxycodone-naïve rats. Ongoing experiments will attempt to determine how intermittent access to oxycodone influences dopamine transmission early in the abstinence period. These observations suggest that exposure to oxycodone engenders distinct behavioral phenotypes, with potentially similar underlying dopamine dynamics.

Disclosures: K.R. Samson: None. **R.A. España:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Relation among potential measures of morphine vulnerability in rats

Authors: *W. WHITE[1], I. M. WHITE[2];
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Abstract: When rats are given 5.0 mg/kg morphine, they show considerable individual differences in the magnitude of their change in activity 12 to 24 hours later: Most animals show a substantial reduction in activity (acute withdrawal sensitive), while others show virtually no change (acute withdrawal insensitive), compared to saline control. The intensity of acute withdrawal following drug administration could be an important determinant of abuse liability, and so identifying predictors of the magnitude of acute withdrawal may be important. The present study continued to assess the predictive relation between potential measures of drug vulnerability and acute withdrawal intensity. Adult male Wistar rats were run on several behavioral procedures thought to yield measures predictive of drug vulnerability, and potentially of acute withdrawal intensity. These measures included percent of time spent in a closed as opposed to an open arm of an elevated maze (a measure of caution); distance moved in a novel open field (a measure of exploration); and degree of conditioning to a CS in a sign/goal tracking task (a measure of sign responsiveness). In addition, to assess aspects of prior morphine receipt that might predict intensity of acute withdrawal, subjects received different doses of morphine (1.25, 2.5, or 5.0 mg/kg), acutely or repeatedly, and the locomotor response to these doses was assessed during the first several hours following treatment (short-term period) and from hours 12 to 24 post treatment (acute withdrawal period). We describe only a couple of the more suggestive relations obtained. Though none of the behavioral measures was significantly correlated with withdrawal intensity, high exploration predicted low short-term locomotor activity to higher morphine doses. In addition, a lower short-term response to higher doses of morphine predicted a more intense acute withdrawal. Overall, results suggested that a behavioral measure of morphine's short-term inhibitory effect, such as a measure of arouse-ability, might predict morphine acute withdrawal and morphine vulnerability.

Disclosures: W. White: None. **I.M. White:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1SC3GM130430-01

Title: Environmental enrichment facilitates early abstinence from heroin in male and female rats

Authors: E. D. BARRERA[1], P. TIMKEN[1], E. LEE[1], H. GOLDSTEIN[1], D. PARASRAM[1], A. VASHISHT[2], *R. RANALDI[1,2];
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Abstract: Opioid dependence is a chronic public health issue. Although pharmacological and behavioral treatments are effective in promoting short-term abstinence they are still largely ineffective in preventing relapse. Environmental enrichment (EE), when implemented non-concurrent with drug exposure, has been shown to diminish drug-seeking in rodent models of cue-induced reinstatement. We have previously demonstrated that EE can significantly reduce cue-induced heroin seeking in male and female rats after periods of craving incubation. Here we tested whether these beneficial effects of EE extend to true (self-initiated) abstinence in males and females. We trained male (N=13) and female (N=16) rats to self-administer intravenous heroin (0.05 mg/kg/infusion) during 3-h sessions, under an FR1 schedule of reinforcement where contingent lever presses were followed by the presentation of a light cue and heroin infusion. After 18 self-administration sessions the rats were randomly assigned to housing; half were placed into EE and the other half into non-EE housing where they stayed for the remainder of the experiments. Thereafter, rats began the abstinence phase. Here 2/3 of the metal rod flooring adjacent to the levers was electrified and the other 1/3 consisted of a no-shock zone. Rats could self-administer heroin in 35-min daily sessions under an FR2 schedule of reinforcement. The current intensity was increased daily by 0.04 mA if the drug-paired lever was pressed in the previous session. This continued daily until abstinence criteria - 3 consecutive sessions without drug-paired lever presses - were met. This was followed by a 35-min cue-induced relapse test during which a non-contingent light-cue was presented every 5 min and current strength was set at 25% of the established abstinence current. Rats in the EE condition reached abstinence criteria in significantly fewer sessions than those in the non-EE condition. Also, abstinence criteria were met significantly earlier in females than males. Finally, cue-induced relapse was significantly higher in females compared to males regardless of housing condition. Our findings provide evidence for the therapeutic efficacy of enrichment in facilitating drug-abstinence and elucidate sex differences in cue-induced relapse that may aid in the development of treatments focusing on relapse prevention.

Disclosures: E.D. Barrera: None. **P. Timken:** None. **E. Lee:** None. **H. Goldstein:** None. **D. Parasram:** None. **A. Vashisht:** None. **R. Ranaldi:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R21DA040777
NIDA Grant R01DA047967

Title: Interleukin-1 receptor-associated kinase 4 (IRAK4) in the nucleus accumbens regulates opioid-seeking behavior

Authors: *R. WU, J. LIU, J. VU, D. M. DIETZ, J.-X. LI;
Univ. at Buffalo, Buffalo, NY

Abstract: Opioid addiction remains a severe health problem. While substantial insights underlying opioid addiction have been yield from neuron-centric studies, the contribution of non-neuronal mechanisms to opioid-related behavioral adaptations has begun to be recognized. Toll-like receptor 4 (TLR4), a pattern recognition receptor, has been widely suggested in opioid-related behaviors. Interleukin-1 receptor-associated kinase 4 (IRAK4) is a kinase essential for TLR4 responses, However, the potential role of IRAK4 in opioid-related responses has not been examined. Here, we explored the role of IRAK4 in cue-induced opioid-seeking behavior in male rats. We found that morphine self-administration increased the phosphorylation level of IRAK4 in the nucleus accumbens (NAc) in rats; the IRAK4 signaling remained activated after morphine extinction and cue-induced reinstatement test. Both systemic and local inhibition of IRAK4 in the NAc core attenuated cue-induced morphine-seeking behavior without affecting the locomotor activity and cue-induced sucrose-seeking. In addition, inhibition of IRAK4 also reduced the cue-induced reinstatement of fentanyl-seeking. Our findings suggest an important role of IRAK4 in opioids relapse-like behaviors and provide novel evidence in the association between innate immunity and drug addiction.

Disclosures: R. Wu: None. **J. Liu:** None. **J. Vu:** None. **D.M. Dietz:** None. **J. Li:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA012513-22 (PWK)

NIDA Grant T32DA007288-30 (BNK)

Title: Cell-type specific ventral pallidum regulation of heroin seeking and refraining behavior

Authors: *B. N. KUHN[1], E. DERESCHEWITZ[2], A. M. KALASKY[2], P. W. KALIVAS[3]; [1]Neurosci., [2]Med. Univ. of South Carolina, Charleston, SC; [3]Neurosci. Res., Med. Univ. S Carolina, Charleston, SC

Abstract: Substance use disorder is a chronic neuropsychiatric disorder characterized by compulsive drug seeking and relapse, in spite of attempts to remain abstinent and refrain from drug use. While significant scientific effort had been put into understanding the neurobiological basis of addiction-related behaviors, less has been done to disentangle the neural circuitry underlying the motivation for drug seeking versus refraining (i.e. withholding drug seeking). Recent work has shown that the dorsolateral ventral pallidum (dlVP) mediates both drug-seeking and refraining behavior, but does so in a cell-type specific manner following cocaine self-administration training. In the current study, transgenic mouse lines expressing Cre recombinase selectively in either glutamate, GABA or enkephalin cells were used. A cre-dependent stimulatory DREADD (Designer Receptors Exclusively Activated by Designer Drugs) was injected into the dlVP of male and female mice, following which mice underwent heroin self-administration training for 10 days. Next, a 1-week forced abstinence period was imposed, after which extinction training (12 sessions) commenced. A test for cue-induced reinstatement followed extinction training. Cell-specific chemogenetic stimulation of dlVP cells occurred during late extinction training and during a test for cue-induced reinstatement using a within-subject design. Contrary to previous finding using cocaine self-administration, stimulation of dlVP glutamate cells had no effect on heroin seeking during extinction training; however, akin to work using cocaine, stimulation did attenuate cue-induced reinstatement of heroin-seeking behavior. Conversely, stimulation of dlVP GABA and enkephalin cells enhanced cue-induced reinstatement of drug-seeking behavior, but did not affect behavior during extinction training as was shown with work using cocaine. These results demonstrate that dlVP cell specific regulation of drug seeking and refraining is conserved across drug classes, albeit with subtle differences. Ongoing work using the same mouse lines are employing an intersectional chemogenetic approach to further dissect cell-type specific circuitry mediating heroin-refraining and seeking behavior. Specifically, the effects of selective stimulation of the glutamatergic projection from the dlVP to the lateral hypothalamus, and the GABAergic projection from the dlVP to the subthalamic nucleus are underway. Taken together, these data will advance our understanding of the cell-type specific dlVP pathways that may contribute to attenuating drug relapse.

Disclosures: B.N. Kuhn: None. **E. Dereschewitz:** None. **A.M. Kalasky:** None. **P.W. Kalivas:** None.

Poster

P757. Opioids: Reinforcement, Seeking, and Reinstatement IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P757.10

Topic: G.09. Drugs of Abuse and Addiction

Title: Characterization of the rat nucleus accumbens transcriptome after acute injection or chronic self-administration of morphine using single nuclei RNAseq

Authors: *B. C. REINER[1], Y. ZHANG[2], E. DAVILA PEREA[1], J. BEN NATHAN[1], K. RAGNINI[1], T. N. FERRARO[3], W. BERRETTINI[1], H. D. SCHMIDT[1], R. CRIST[1]; [1]Psychiatry, [2]Biobehavioral Hlth. Sci., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [3]Biomed. Sci., Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: Opioid use causes broad transcriptomic changes across many regions of the brain. In animal models, acute injection and self-administration result in distinct gene expression profiles suggesting differences between the proximal effects of opioids and neural adaptations to chronic exposure. However, studies identifying how these phenotypes affect the transcriptome of neuronal cell types is limited. The goal of the current study is to compare the transcriptomic changes following acute injection and chronic self-administration of morphine using single nucleus RNA sequencing (snRNAseq). Male Brown Norway rats (n=5/group) were treated with a single intraperitoneal injection of morphine or saline. A second cohort of rats (n=6/group) were given surgically-implanted jugular catheters and assigned to either 10 days of morphine self-administration in a FR1 operant conditioning model or yoked saline control. Brains from all animals were snap frozen and nucleus accumbens samples were punched. SnRNAseq libraries were generated using the 10x Genomics Chromium Controller and sequenced on a NovaSeq 6000. The R package Seurat was used for quality control and to cluster nuclei based on transcriptomic data. Identities of clusters were determined using expression of known cell-specific marker genes. The R package MAST was used to analyze differential expression between cases and control in each cluster separately for the acute and chronic groups. Ingenuity Pathway Analysis and FUMA were used for analysis of canonical pathways and upstream regulators. 27 transcriptomically distinct clusters of nuclei were identified. In these clusters, acute morphine injection resulted in 1,106 differentially expressed genes (DEGs), compared to 2,453 DEGs in the chronic self-administration model. The majority of DEGs for both treatment paradigms were observed in oligodendrocytes, astrocytes, and D1 and D2 medium spiny neurons. However, the specific DEGs identified in these cell types were largely unique to either acute or chronic morphine treatment. Cell type-specific differential expression was observed for numerous genes previously linked to opioid phenotypes (e.g. Oprm1, Hspa4, Pde10a). Pathway analysis revealed cell type-specific activity changes. These data indicate that both acute and chronic morphine exposure result in notable cell type-specific transcriptomic changes in the nucleus accumbens, and identification of DEGs and activated pathways with prior connections to opioids provides validity for our findings and suggests that future work must focus on the roles specific cell types play in opioid use disorder.

Disclosures: B.C. Reiner: None. **Y. Zhang:** None. **E. Davila Perea:** None. **J. Ben Nathan:** None. **K. Ragnini:** None. **T.N. Ferraro:** None. **W. Berrettini:** None. **H.D. Schmidt:** None. **R. Crist:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.01

Topic: H.07. Long-Term Memory

Support: DARPA N66001-14-C-4016
WFBMC Dept. of Neurosurgery
NIBIB

Title: Coordinating Memory with Stimulation: Strength And Congruency Of Stimulation Patterns In Comparison To Native Neural Codes

Authors: *B. ROEDER[1], X. SHE[2], M. RILEY[1], B. MOORE[2], D. SONG[2], T. BERGER[2], S. DEADWYLER[1], R. HAMPSON[1];
[1]Wake Forest Sch. of Med., Winston Salem, NC; [2]USC, Los Angeles, CA

Abstract: Human memory is subject to decline and loss due to disease and injury. Social and economic costs of Alzheimer's Disease, Traumatic Brain Injury, Stroke and other conditions are billions of dollars per year. Development of a neural prosthetic for human memory requires understanding how hippocampal stimulation interacts with natural or "native" neural codes present during stimulation. Previously, this laboratory used a combined Delayed-Match-to-Sample+Delayed Recognition (DMS-DR) task to study how effectively different stimulation patterns modulate memory performance. The results of this task also allow exploration of how stimulation and native neural codes interact.
The standard DMS-DR task presents Sample, Match and Nonmatch images from five categories (animal, building, plant, tool, vehicle). However, if the original (Sample) image is ambiguous (i.e. a grey square instead of a category image), there is minimal category-specific native neural activity, and Match phase choices are randomized. In those cases, stimulating with category patterns during the Sample phase increases the rate that categories associated with the selected stimulation pattern in the DMS match phase (Deadwyler et al, 698.05, SFN2019, Chicago, Il), suggesting that stimulation "writes" the stimulated category information into the hippocampus. Furthermore, random stimulation produces either no change or a slight decrease in memory performance (Hampson et al. J. Neural Eng, 2018, 15:036014).
To further assess the interaction or interference between stimulated and native neural codes, we examined DMS+DR tasks where the categories of the Sample image matched the stimulation pattern. When stimulation patterns were modeled from a subject's neural recordings, we

observed over 13% of category and patient combinations had increased memory performance, just over 79% had no change, and 7% had decreased performance. When patterns were derived from models constructed across one or more other subjects, 8% of category and patient combinations had increased memory performance, 84% had no change, and 8% had decreased performance.
This interaction suggests that two components dictate how a stimulation pattern will interact with a native code, the strength of the pattern and how congruent the pattern is to native codes. We propose that "strength" results from the relationship of neurons active natively to stimulation activated neurons. "Congruence" indicates how well stimulated patterns emulate native patterns. Further exploration of pattern congruency and strength are important to the ability to create, select, and adjust patterns for desired neural prosthetic stimulation outcomes.

Disclosures: B. Roeder: None. **X. She:** None. **M. Riley:** None. **B. Moore:** None. **D. Song:** None. **T. Berger:** None. **S. Deadwyler:** None. **R. Hampson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Braingrade, Inc..

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.02

Topic: H.07. Long-Term Memory

Support: DARPA N66001-14-C-4016
WFBMC Dept. of Neurosurgery
NIBIB

Title: Coordinating Memory with Stimulation: Off-Target Application of Content Codes

Authors: *R. HAMPSON[1], B. ROEDER[1], M. RILEY[1], X. SHE[2], B. MOORE[2], D. SONG[2], T. W. BERGER[2], S. DEADWYLER[1];
[1]Wake Forest Sch. of Med., Winston Salem, NC; [2]Biomed. Engin., USC, Los Angeles, CA

Abstract: In the adjacent poster, we studied the strength and congruence of hippocampal stimulation as it affects Human memory. Development of a neural prosthetic for human memory also requires an understanding of the effects of stimulation that is deliberately noncongruent with natural or "native" neural codes already present at the time of stimulation.
Stimulation during a combined Delayed-Match-to-Sample+Delayed Recognition (DMS+DR) task that utilized images in five categories (animal, building, plant, tool, and vehicle) resulted in both increases and decreases in memory when the category pattern used to stimulate did not match the category of the sample image (Hampson et al, 698.04, SFN 2019, Chicago, Il). For

these studies we modeled stimulation patterns from neural recordings taken while subjects performed a DMS task with Sample pictures categorized as containing animal, building, plant, tool or vehicle images. While it was intended that the modeled "codes" were specific to only the desired category, many images contained "shared content," such as plant in pictures of both animals and buildings. Thus, there may be overlap between modeled codes for different categories.
On tasks where the stimulated Sample code was deliberately "noncongruent" (i.e. did not Match the category of the stimulation pattern) and the patterns were specifically derived form that individual subject's own neural recordings, we observed that over 10% of category and patient combinations had increased memory performance, approximately 76% of combinations had no change in performance, and 13% had decreased memory performance. When the stimulation codes were derived from neural recordings from one or more other (donor) subjects; over 10% of category and patient combinations had increased memory performance, 84% of combinations had no change in performance, and 5% had decreased memory performance.
Decreases in DMS+DR performance suggests noncongruent codes that are sufficiently strong to interfere with native codes. In contrast, increases in performance may result from "weak" codes, however, that is unlikely due to prior results of no change (or decrease) in performance due to random stimulation pattern. More likely, there is overlap in between codes for the separate categories, which can result in improved performance, particularly where the stimulated code is able to "write-in" at least partial category information as with ambiguous Sample trials.

Disclosures: **R. Hampson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Braingrade, Inc.. **B. Roeder:** None. **M. Riley:** None. **X. She:** None. **B. Moore:** None. **D. Song:** None. **T.W. Berger:** None. **S. Deadwyler:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.03

Topic: H.07. Long-Term Memory

Support: DARPA N66001-14-C-4016

Title: Coordinating Memory with Stimulation: Decoding Spatio-Temporal Patterns of Spikes with Small Sample Size Using a Double-Layer Multi-Resolution Classifier

Authors: *X. SHE[1], B. M. ROEDER[4], G. NUNE[1], B. LEE[2], S. SHAW[6], A. NGUYEN[6], C. HECK[1], C. LIU[1], S. A. DEADWYLER[5], R. E. HAMPSON[5], T. W. BERGER[3], D. SONG[3]; [1]USC, Los Angeles, CA; [2]USC, San Marino, CA; [3]Biomed. Engin., USC, Los Angeles, CA;

[4]Neurosci., [5]Wake Forest Sch. of Med., Winston Salem, NC; [6]Rancho Los Amigos Rehabil. Ctr., Los Angeles, CA

Abstract: We build a double-layer multiple temporal-resolution (multi-resolution) classifier for decoding single-trial behavioral/cognitive information from spatio-temporal patterns of spikes. The structure of this classifier is designed specifically for solving the highly underdetermined estimation problem caused by high-dimensional spike patterns, the small sample size, and taking into account the unknown optimal temporal resolution for decoding spikes. The double-layer multi-resolution classifier consists of two layers. In the first layer, to solve the underdetermined problem resulting from the small sample size and the very high dimensional input and output signals, B-spline basis functions are applied to reduce dimensionality; *L*1-regularized logistic regression (LASSO) classifiers are used to achieve sparse model estimation. Multiple classifiers with different B-spline resolutions are employed to include a wide range of temporal resolutions of neural signals. Each classifier serves as a base-learner to classify spatio-temporal patterns with a given temporal resolution. A bagging-based sampling strategy is used to further reduce the estimation variances of these classifiers. In the second layer, another LASSO classifier takes outputs of first-layer classifiers as inputs to make the final output prediction. This ensemble classifier serves as a meta-learner that combines multiple temporal resolutions to classify the spatio-temporal patterns of spikes. Results in both synthetic and experimental data show that this classifier can accurately decode high-dimensional spike patterns using a small number of instances. The double-layer classifier consistently outperforms the single-layer classifier by utilizing multi-resolution spatio-temporal features of spike patterns in classification. The decoding classifier also provides signature functions that represent spatio-temporal characteristics of spike patterns most relevant to classifications.

Disclosures: X. She: None. **B.M. Roeder:** None. **G. Nune:** None. **B. Lee:** None. **S. Shaw:** None. **A. Nguyen:** None. **C. Heck:** None. **C. Liu:** None. **S.A. Deadwyler:** None. **R.E. Hampson:** None. **T.W. Berger:** None. **D. Song:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.04

Topic: H.07. Long-Term Memory

Support: DARPA N66001-14-C-4016
WFBMC Dept. of Neurosurgery
NIBIB

Title: Coordinating Memory with Stimulation: Hippocampal Stimulation in the Injured Brain

Authors: *M. RILEY[1], B. M. ROEDER[1], X. SHE[2], B. MOORE[2], D. SONG[2], T. W. BERGER[2], S. A. DEADWYLER[1], R. E. HAMPSON[1];
[1]Physiology/Pharmacology, Wake Forest Sch. of Med., Winston Salem, NC; [2]Biomed. Engin., USC, Los Angeles, CA

Abstract: Beneficial effects from experimental treatments are not distributed equally among all participants of the study nor are approved treatments fully beneficial for all patients. Some patients see a larger improvement of outcomes or a larger decrease in negative symptoms while others may see barely any effects. Our lab has shown that encoding of stimuli in memory could be enhanced or impaired using hippocampal stimulation based upon different categories in human models (Hampson et al. J. Neural Eng, 2018, 15:036014).
Recently we tested different models used to influence short-term memory in human hippocampal ensemble activity during performance of a task for specific categories of visual stimuli that would either match the category or would match to a different category (See Roeder et al, and Hampson et al, this session). In human patients undergoing Phase II invasive monitoring for intractable epilepsy, recordings from an initial DMS (training) session were employed to determine neuron firing patterns for five categories of images; animal, building, plant, tool, and vehicle.
We used the Multiple-in-Multiple-Out (MIMO), the Memory Decoding Model (MDM), and a combination of both models to test five different image content categories and stimulated using patterns positively and negatively correlated with each category. Calculation of specific stimulation patterns prior to the stimulation session allowed to identify CA1 and CA3 neurons as stimulation targets.
Of our subject population, a subset had previously undergone either a repeated and/or mild-to-moderate brain injury (RMBI) or a traumatic brain injury (TBI). The subject's medical history was unknown to the experimenters while conducting the experiment. When examined compared to control subjects, these subjects showed a much larger increase when using positive stimulation patterns and a much larger decrease when using negative stimulation patterns. Each model as well as the combined stimulation from both models showed these same behavioral changes on our DMS task, indicating that this type of hippocampal stimulation was effective at improving memory performance.
These results provide evidence that models for memory stimulation are more beneficial for previously-injured patients in aiding when performing cognitive tests than control patients who had not suffered a brain injury previously. Additionally, they emphasize the justification for continuing to develop this technology to aid people who have suffered brain injuries.

Disclosures: M. Riley: None. **B.M. Roeder:** None. **X. She:** None. **B. Moore:** None. **D. Song:** None. **T.W. Berger:** None. **S.A. Deadwyler:** None. **R.E. Hampson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Braingrade, Inc..

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.05

Topic: H.07. Long-Term Memory

Support: NIH/NCATS UL1TR003015
NIH/NCATS KL2TR003016

Title: Temporal context modulates encoding and retrieval of overlapping events

Authors: *D. E. SMITH, I. L. MOORE, N. M. LONG;
Univ. of Virginia, Charlottesville, VA

Abstract: Interference between overlapping events occurs due to a tradeoff between encoding the present event and retrieving the past, overlapping event. Temporal information - 'when' something occurred - is a defining feature of episodic memory and can cue retrieval of a past event. However, the influence of temporal overlap, or proximity in time, between events on interference is unclear. To test the effect of temporal overlap on interference, we conducted a human scalp electroencephalography (EEG) study in which subjects were explicitly directed to either encode the present event or retrieve a past, overlapping event. Critically, we manipulated temporal overlap between events by varying the distance between pairs of objects, whereby the closer two objects are in time, the greater their temporal overlap. We find that greater temporal overlap between two objects leads to selective interference and impaired memory for past objects when the top-down goal is to encode the present object. We used pattern classification analyses to measure the extent to which participants engaged a 'retrieval state' -- a brain state distinct from encoding in which attention is directed internally. We find that greater temporal overlap leads to automatic retrieval of a past event, independent of top-down goals to either encode or retrieve. The retrieval state that we observe likely reflects a general retrieval mode, rather than retrieval success or effort. Together, these findings provide insight into the role of temporal overlap on interference and memory formation.

Disclosures: D.E. Smith: None. **I.L. Moore:** None. **N.M. Long:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.06

Topic: H.07. Long-Term Memory

Support: R01 MH069456
James S. McDonnell Foundation
NSF GRFP

Title: Episodic encoding in the infant brain revealed through subsequent memory

Authors: *T. S. YATES[1], C. T. ELLIS[1], N. B. TURK-BROWNE[1,2];
[1]Psychology, [2]Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Episodic memories from infancy and early childhood are rare later in life, raising questions about whether they were encoded in the first place. Such infantile amnesia in adults and behavioral tasks in children suggest that episodic memory abilities, and the hippocampal circuits they require, may not mature until as late as middle childhood. However, recent neurophysiological studies of young rodents and neuroimaging studies of human infants and toddlers suggest that the infant hippocampus is functional and contributes to some forms of early learning. Whether and how the hippocampus supports episodic encoding in infants under two years has never been tested. We collected fMRI data from awake infants while they performed a variant of the subsequent memory design commonly used in adult cognitive neuroscience. Encoding trials presented an image of a face, object, or scene on an attention-getting psychedelic background. Test trials were inserted continuously into the trial sequence at study-test lags of 30-90 seconds. These trials displayed two images, the old item and a new item from the same category, in a visual paired comparison task. Looking time to the old and new items was coded offline to quantify an item-specific novelty/familiarity preference, as a marker of recognition memory strength. We then back-sorted the encoding trials based on this later test preference, to identify brain regions whose activity during encoding predicted subsequent novelty/familiarity. To date, a partial sample of 10 infants has been collected. We first contrasted task activation for trials containing faces, objects, and scenes, and found robust and selective neural responses in infant visual cortex that aligned with category-selective regions from an adult meta-analysis. We then used a parametric model to relate encoding activity to the proportion of looking to the old item at test. Preliminary results indicate involvement of brain regions in infants that have been linked to episodic memory in adults, including in the medial temporal lobe. This ongoing research contributes to an emerging body of evidence that infants may have more robust memory abilities and more developed memory systems than typically credited.

Disclosures: T.S. Yates: None. **C.T. Ellis:** None. **N.B. Turk-Browne:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.07

Topic: H.07. Long-Term Memory

Support: NIH/NCATS UL1TR003015
NIH/NCATS KL2TR003016

Title: Multivariate decoding of memory brain states across the lifespan

Authors: *I. L. MOORE, 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 retrieve prior knowledge. A retrieval state is neurally distinct from and trades off with an 'encoding state,' a brain state in which attention is focused externally. To test our hypothesis, we conducted a scalp electroencephalographic (EEG) experiment in which younger and older adult participants explicitly engaged in encoding and retrieval states. Specifically, participants viewed a list of common objects (e.g. bench, apple; List 1 objects), then viewed a list of non-identical, but categorically related objects (e.g. a new bench, a new apple; List 2 objects). On each List 2 trial, participants were instructed to either encode the current List 2 object (e.g. the new bench) or retrieve the categorically related List 1 object (e.g. the old bench). We measured memory for all objects in a two-alternative forced choice recognition task. Both younger and older adults showed better memory for List 2 objects paired with the 'encode' compared to the 'retrieve' instruction. Numerically, older adults showed worse List 2 memory compared to younger adults, supporting the interpretation that older adults are more likely to engage in retrieval during List 2. We used multivariate pattern classification to assess the extent to which participants engaged in encoding and retrieval states. We find above chance classification for both younger and older adults, meaning that distinct electrophysiological signatures support encoding vs. retrieval. We assessed classifier evidence -- a continuous measure of how confident the classifier is that the current trial is an 'encode' or 'retrieve' trial -- across the List 2 stimulus interval (2000 ms). We find that dissociations between encode and retrieve trials occur earlier in the stimulus interval for older compared to younger adults. Together these findings suggest that engagement of encoding and retrieval states, specifically in terms of temporal dynamics, differs across the lifespan and may relate to episodic memory deficits observed in healthy aging.

Disclosures: I.L. Moore: None. **N.M. Long:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.08

Topic: H.07. Long-Term Memory

Support: R01 MH069456, James S. McDonnell Foundation

Title: Automated segmentation outperforms manual segmentation of the infant hippocampus

Authors: *J. T. FEL, C. T. ELLIS, N. B. TURK-BROWNE;
Psychology, Yale Univ., New Haven, CT

Abstract: The hippocampus, critical for learning and memory, undergoes substantial changes in the first years of life. Investigating the developmental trajectory of hippocampal structure and function requires an accurate method for segmenting this region from anatomical MRI scans. Although manual segmentation is often regarded as the "gold standard" approach, at least in adults, it is time-consuming, requires considerable expertise, and relies on variable landmarks across labs. These drawbacks have fueled the pursuit of automated segmentation methods. However, little is known about the reliability of these automated protocols in tracing the hippocampus of human infants, particularly given the greater noise and lower quality of anatomical scans in infants. Here we evaluate the efficacy of different approaches for automated hippocampal segmentation in infants. As part of an awake, task-based protocol, we collected short T1-weighted anatomical scans from 42 sessions with infants aged 4 to 23 months. For each session, two trained tracers manually segmented the hippocampus as a benchmark measurement of inter-rater reliability. Four different automated methods for hippocampal segmentation were considered: adult anatomical average template, infant anatomical average template (derived from infant data linearly and nonlinearly aligned into standard space), FreeSurfer software, and Automated Segmentation of Hippocampal Subfields (ASHS) software. Although the adult anatomical template failed to approximate manual segmentation data accurately, the infant anatomical template adequately predicted the infant data of both tracers. FreeSurfer generated moderately accurate segmentations that approached the average inter-rater reliability of manual segmentations; however, this came at the cost of over-estimating hippocampal volumes. ASHS generated the most reliable and least biased hippocampal segmentations, even exceeding manual inter-rater reliability. ASHS was successful despite being trained on T1-weighted anatomical scans that were low-quality and noisy by conventional standards. Hence, automated methods can provide accurate, generalizable segmentations in infants that make it possible to accelerate and improve the study of the developing hippocampus.

Disclosures: J.T. Fel: None. **C.T. Ellis:** None. **N.B. Turk-Browne:** None.

Poster

P816. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P816.09

Topic: H.07. Long-Term Memory

Title: Can You Scan Lefties? The Effect of Handedness on fMRI Activation in a Recognition Memory Paradigm

Authors: L. GOULDING, **I. HAMM**, *B. KIRWAN;
Brigham Young Univ., Provo, UT

Abstract: About 11% of the world population is left-handed, a significant minority of the potential research participant pool for functional MRI (fMRI) studies. However, convention in fMRI research dictates that these participants should be excluded from analysis due to evidence that left-handed participants may have a different pattern of lateralization of specific functions than right-handed participants. This could alter results in fMRI studies, as different areas of the brain may be activated by the same task in these individuals. This hypothesis has been tested for some cognitive processes, but has yet to be thoroughly tested in memory research despite the frequent exclusion of left-handed individuals from research projects. The current study investigates the lateralization differences between N=26 left- and N=27 right-handed participants while they performed an encoding and recognition memory task for words and faces. Additionally, we measured participants' laterality index by administering a semantic fluency task. At encoding, we found one cluster of activation in the right posterior cingulate cortex, where there was a handedness by subsequent memory effect interaction collapsing across words and faces. There was no interaction between words and faces and handedness at encoding. At retrieval, we found no interaction between handedness and trial outcome. Conversely, there were several clusters that demonstrated a significant interaction between stimulus type (words, faces) and handedness including the left inferior frontal gyrus, left angular gyrus, right angular gyrus, and left superior medial gyrus. In order to measure if including left-handed individuals in fMRI studies would alter results significantly, we calculated memory effects in a priori ROIs for the right-handed participants only, and then examined the effect of substituting in progressively more left-handed participants for right-handed participants. We found that subsequent memory effects at encoding were not reduced by adding in left-handed participants. However, at retrieval, significant memory effects diminished in the bilateral precuneus for faces and in the left hippocampus for words when substituting in six and five left-handed participants, respectively. These findings suggest that while the blanket exclusion of left-handed individuals in memory research is not warranted, exclusion for research in specific ROIs may be justified.

Disclosures: L. Goulding: None. **I. Hamm:** None. **B. Kirwan:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.01

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant 5R01MH120125-02
Foundation of Hope
NIH Grant 5 T32 GM 67553-15

Title: Common genetic variation modulates lithium-induced neural progenitor proliferation

Authors: *B. D. LE[1], J. M. WOLTER[2], N. MATOBA[2], J. M. VALONE[1], A. L. ELWELL[2], N. AYGÜN[1], D. LIANG[1], M. J. LAFFERTY[1], K. P. COURTNEY[2], J. PIVEN[3], M. J. ZYLKA[4], J. L. STEIN[2];
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Abstract: Neural progenitor cell proliferation during human fetal development shapes the structure of the cerebral cortex. Dynamic regulation of gene expression orchestrates this process and is tuned both by inherited genetic effects and exposure to developmentally relevant stimuli. Lithium, a common neuropsychiatric treatment also found ubiquitously at trace amounts, is one such stimulus that impacts neural progenitor proliferation through the Wnt signaling pathway. We measured the effects of therapeutically relevant levels of lithium on proliferation in 80 genetically diverse primary human neural progenitor cell-lines. Proliferation was measured with a flow cytometry based assay that fluorescently labeled newly synthesized DNA in actively dividing cells via EdU incorporation. Low (0.75 mM) and moderate (1.5 mM) lithium exposures additively increased the proportion of proliferating cells, but a stronger non-therapeutic dose (3 mM) decreased proliferation. We performed a genome-wide association study (GWAS) to identify common genetic variants modulating the proliferative response to lithium. Comparison of GWAS signals show that genetic contributions to proliferation differ between baseline and stimulated cellular states and are stimulus and dose specific. One locus, associated with change in the number of proliferating neural progenitors in response to 1.5mM lithium, colocalized with GWAS signals for schizophrenia and bipolar disorder risk on chr3p21.1. Here we identified a single gene, *GNL3*, that exhibited lithium-responsive gene expression over a 2-24h time course following treatment. The allele associated with increased lithium-induced proliferation is also associated with increased *GNL3* expression from gene expression quantitative loci in GTEx brain tissue data. To investigate the role of *GNL3* in lithium induced proliferation, we targeted the *GNL3* promoter with dCas9-KRAB constructs and found that suppressing *GNL3* expression blunted increases in proliferation induced by lithium, showing that *GNL3* expression levels modulate proliferation responses to lithium. Our results demonstrate how a proliferation response GWAS can identify loci where genetic variation alters stimulus-specific regulation of gene expression within a key cell-type and developmental time period. Colocalization of this lithium-responsive proliferation locus and GWAS loci for complex traits like Bipolar disorder suggests a shared genetic basis for fetal neural progenitor proliferation responses and neuropsychiatric disorder risk and invites exploration into the role of *GNL3* modulating responses to lithium treatment in adult neural progenitor cells.

Disclosures: B.D. Le: None. **J.M. Wolter:** None. **N. Matoba:** None. **J.M. Valone:** None. **A.L. Elwell:** None. **N. Aygün:** None. **D. Liang:** None. **M.J. Lafferty:** None. **K.P. Courtney:** None. **J. Piven:** None. **M.J. Zylka:** None. **J.L. Stein:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01AA024659

Title: Sex differences in gliogenic markers in neural stem cells and their extracellular vesicles are decreased by ethanol exposure

Authors: *A. H. MAHNKE[1,2], A. MILLER[1], V. YANG[1], D. D. CHUNG[1], R. C. MIRANDA[1,2]; [1]Dept. of Neurosci. and Exptl. Therapeut., [2]Women's Hlth. in Neurosci. Program, Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: Prenatal alcohol exposure (PAE) can result in growth and neurodevelopmental deficits, termed ‘fetal alcohol spectrum disorders’, that are estimated to affect 1-5% of children in the US. PAE can teratogenically reprogram neural stem cells to favor premature maturation and decrease self-renewal. The transcription factor nuclear factor I A (Nfia) has been shown to act as a gliogenic switch, inducing the formation of astrocytes from stem cells, and may mediate PAE-induced premature maturation of neural stem cells. Here, we investigated the effect of ethanol exposure on the expression of Nfia in neural stem cells. These cells were derived from murine GD 12.5 dorsal neuroepithelium and propagated in vitro as non-adherent neurospheres. Fetal tissues were genotyped at the time of collection to allow for the creation and propagation of sex-segregated neurosphere cultures. Neurospheres were exposed to ethanol in their mitogenic media for 5 days and subsequently collected for bulk RNA sequencing and LC-MS/MS-based proteomics. Extracellular vesicles (EVs) were also collected from cell culture media at day 5 for proteomics analysis. Using RNAseq we found that exposure to low dose (60 mg/dL) ethanol significantly decreased glial marker *Gfap* mRNA expression, contrary to our hypothesis that ethanol exposure would increase markers of differentiation. There was a sex difference in *Gfap* expression with male neurospheres trending towards higher expression of *Gfap* mRNA compared to female neurospheres. While there were no differences in *Nfia* mRNA expression, proteomics analysis showed that males neurospheres had significantly higher levels of Nfia protein and glial marker Glast/Slc1a3 across all ethanol treatment groups. Nfia protein was also present in neurosphere-derived EVs and, surprisingly, female neurosphere-derived EVs had 2.3-fold the Nfia protein level of male neurosphere-derived EVs. This sex difference in EV Nfia

enrichment decreased with increasing levels of ethanol exposure such that following exposure to a chronic use-like ethanol dose (320 mg/dL), female derived-EVs had 1.5-fold the Nfia protein as male derived-EVs. Together, these data indicate baseline sex differences in neurosphere expression of gliogenic markers and exposure to high levels of ethanol decreases this difference. Intriguingly, ethanol's effects on neural stem cell maturation may occur both through direct changes to the cellular proteome and indirectly through EV transcription factor cargo. Further work is needed to identify the mechanisms of sex-specific trafficking of Nfia to EVs.

Disclosures: A.H. Mahnke: None. **A. Miller:** None. **V. Yang:** None. **D.D. Chung:** None. **R.C. Miranda:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.03

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant F31AA028446
NIH Grant R01AA024659
NIH Grant R01HD086765

Title: Ethanol Effects on the Proteome and Transcriptome of Fetal Neural Stem Cell and their Extracellular Vesicles

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Abstract: Prenatal alcohol exposure (PAE) can alter the growth and maturation of neural stem cells (NSCs), leading to diminished brain growth. NSCs reside in a complex microenvironment rich in sub-200 nanometer-sized extracellular vesicles (EVs), which may traffic protein, lipid, and RNA cargo between cells, as a mode of intercellular communication. Using fetal mouse derived cortical neuroepithelium, cultured ex-vivo as non-adherent neurosphere cultures, we previously found that ethanol exposure resulted in significant elevation of n miR-140-3p, in EVs, which directed NSCs towards an astroglial lineage. For this study, we further investigated the impact of ethanol on the proteome and transcriptome of NSC-EVs by employing quantitative proteomics and transcriptomics to profile the protein and RNA expression across treatment groups of 18 EV and its 18 parent NSCs.

Analyses of EVs identified ~86% of proteins needed for eukaryotic translation initiation, implying that EVs carry with them the ability to translate EV-chaperoned mRNAs in recipient cells. Statistical and pathway overrepresentation analyses showed that moderate ethanol, ~26 mM, resulted in a significant increase in proteins of the Nonsense-Mediated Decay (NMD) pathway in EVs, whereas a higher dose, ~70 mM, resulted in EV overexpression of mitochondrial proteins that constitute a Danger-Associated Molecular Pattern (mito-DAMP) pathway. NMD is a surveillance pathway that reduces errors in gene expression by eliminating premature stop codon-containing mRNA transcripts. Consequently, NMD pathway proteins sequestered in EVs are hypothesized to transfer neuroprotection to cells where the capacity to correct errors in protein translation may be depleted. Eukaryotic cells under high stress, expel mitochondrial proteins to activate 'pattern recognition' receptors and pro-inflammatory responses in target cells. Consequently, mito-DAMPs in EVs from heavy-ethanol-exposed NSCs are predicted to spread inflammation through the NSC microenvironment.
Preliminary analysis shows that that the RNA transcript reads in EV samples is biased towards a higher percentage of introns and other intergenic transcripts and a lower percentage of exons compared to parental NSCs. Combined with our proteomic data showing that EVs contain a high number of RNA-binding proteins, these data suggest that un-spliced RNA transcripts are transcribed and directly chaperoned for packaging into EVs rather than being processed into mature mRNAs. Collectively, these studies identify EVs as a novel source for communicating stress responses, in an ethanol dose-related manner, to cells within the fetal NSC niche.

Disclosures: D.D. Chung: None. **N.P. Collins:** None. **E.A. Payne:** None. **K.T. Le:** None. **M.S. Lai:** None. **N.A. Salem:** None. **S. Weintraub:** None. **R.C. Miranda:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.04

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant 1R21NS097899

Title: Buprenorphine attenuates the growth suppressive effect of methadone in human brain organoid

Authors: *H. YAO[1], W. WU[1], J. WANG[1], G. HADDAD[1,2];
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Abstract: Methadone and Buprenorphine are opioids primarily used for medication-assisted treatment (MAT) for opioid use disorder. Although both drugs can lead to neonatal abstinence

syndrome (NAS) in newborns when used maternally, buprenorphine shows a significantly reduced NAS duration. Our previous work has shown that methadone can inhibit brain organoid growth, decrease synaptic transmission and suppress neural network activities. In this work, we hypothesize that buprenorphine does not affect brain organoid growth as much as methadone. Brain organoids were treated with various dosages of buprenorphine or methadone starting from the formation of embryoid body until the end of the neural induction, proliferation, differentiation and maturation stages (about a month). In the control group, the organoids grew by 30.4±4% in size from day 3 to day 24 in culture but 1 μM methadone blunted this increase to 8.1±3%. At 5 or 10 μM, methadone eliminated any increase and indeed organoids shrunk in size by -38.1±9.3% or -67.7±12.3%, respectively ($n=5$, $p<0.05$ for each group). While in the buprenorphine-treated group (at dosages of 1, 10 or 30 μM), the increase of organoid size did not differ from control. In another group of experiments, we treated organoids with 5 μM methadone plus buprenorphine at dosages of 1, 10 or 30 μM, respectively. Our data show that, while methadone alone suppressed the increase of organoid size by a third of its control rate, the addition of buprenorphine at 1, 10 or 30 μM, restored the increase of organoid size to about 8 to 25% in that duration, from day 3 to day 24 ($n=5$, $p<0.05$ for each group). We conclude that a) buprenorphine does not alter the growth rate of brain organoids as methadone does; and b) buprenorphine can eliminate the suppressive effect of methadone in organoids. These observations may explain the attenuated NAS duration in newborns treated with buprenorphine.

Disclosures: H. Yao: None. **W. Wu:** None. **J. Wang:** None. **G. Haddad:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.05

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01 AA028406

Title: Polysubstance exposure to ethanol and cannabinoids induces synergistic effects on mouse embryonic neural stem cell growth

Authors: *S. ROUZER, A. ADAMS, S. MAVURI, R. MIRANDA;
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Abstract: Prenatal drug exposure is the leading cause of neurodevelopmental disorders worldwide, often occurring prior to pregnancy detection (~1st/early 2nd trimesters). Importantly, this gestational period corresponds with pronounced fetal neural stem cell (NSC) activity. NSCs are multi-potent cells with the ability to self-renew and proliferate, and in utero give rise to most

neurons and glia of the adult brain. Through culturing of these cells in the lab, we can investigate NSC proliferation, differentiation and survival, which is critical for treating developmental and neurodegenerative disorders. Prior research has demonstrated that the introduction of ethanol to cultured cells results in teratogenic reprogramming of NSCs. However, the impact of additional drug co-exposures is yet unexplored, despite the prevalence of simultaneous drug consumption in women of child-bearing age. Therefore, we investigated the effects of drug exposure on NSC growth in vitro using neurosphere cultures from the dorsal neuroepithelium of GD 12.5 C57/Bl6 mice. Neurospheres were exposed to a concentration range of nicotine, ethanol and/or synthetic cannabinoid CP-55940 for 5 days, at which point cells were passaged to drug-free media for future analyses. Using ImageJ/FIJI, we analyzed neurosphere area as an indication of NSC growth. Preliminary analyses revealed an acute increase in neurosphere area following co-exposure to low concentrations of ethanol and CP-55940, mimicking singular, high concentration exposure of either drug. Importantly, following serial passaging of NSCs to assess self-renewal, this synergistic low ethanol-and-cannabinoid effect was reversed, with combined exposure now contributing to reduced neurosphere area, along with a notable decrease in cell survival, compared to drug-naïve controls. Furthermore, nicotine exposure did not alter neurosphere area when combined with ethanol; rather, nicotine independently decreased neurosphere area, but only following multiple cell passages. Together, these data suggest that combined ethanol-and-cannabinoid exposure may uniquely induce premature NSC growth and subsequently stunt the growth/proliferation of future cell progeny. To identify the underlying mechanisms impaired by this embryonic polysubstance exposure, further research will incorporate immunostaining and RNAsequencing of neural growth factors within exposed NSCs.

Disclosures: S. Rouzer: None. **A. Adams:** None. **S. Mavuri:** None. **R. Miranda:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH T32-AA014127
NIH 1R01NS116051-01A1
NIH 5P50AA022534-08

Title: Modeling Fetal Alcohol Spectrum Disorder in Human Cerebral Organoids

Authors: *M. FISCHER[1], C. ROBBINS[1], J. P. WEICK[2];
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Abstract: Fetal alcohol spectrum disorders (FASD) due to gestational alcohol exposure represents one of the most common causes of nonheritable lifelong disability worldwide. The specific mechanisms of alcohol's teratogenicity remain incompletely understood but the convergence of its effects on the developing brain have long been understood to contribute to lifelong deleterious cognitive and behavioral outcomes. In this study we utilized a human stem cell-based model of cortical development to determine the effects of first trimester-equivalent chronic binge alcohol exposure on the differentiation of neuronal cell populations and their progenitors. Significant alterations were discovered to the expression of a number of regionally defined transcription factors, including significant upregulation of the postero-dorsal transcription factor EMX2 and a concurrent downregulation of the antero-ventral marker SIX3 in a self-patterning human cortical organoid (hCO) system. Further single-cell sequencing revealed readily identifiable populations of stem cells, neural progenitors and post-mitotic neurons with a primarily dorsal pallial transcriptional signature in each the presence and absence of alcohol *in vitro*. Reduced complexity of fate determination processes in alcohol-exposed hCOs was determined via MONOCLE3 pseudotime-based lineage tracing analyses. Transcripts associated with the maintenance of pluripotency (ID2/4), the specification of regional cortical identity (EMX1/2, SIX3), and WNT-signaling pathways (RSPO1/2/3) were identified as primary targets of alcohol in developing and mature neuronal cells by each gene expression and protein analyses of organoid cultures. Together these data provide further support for altered cell specification and regional patterning of the developing forebrain as a primary mechanism underlying FASD.

Disclosures: M. Fischer: None. **C. Robbins:** None. **J.P. Weick:** None.

Poster

P038. Effects of Alcohol and Other Substances on Brain Development: Stem Cell Approaches

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P038.07

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant 5R01HD087288-05

Title: Genomic glucocorticoid action in the developing brain

Authors: *K. BERRY[1], U. CHANDRAN[1], F. MU[2], I. ROGATSKY[3], D. DEFRANCO[4,3]; [1]Univ. of Pittsburgh Ctr. For Neurosci., Pittsburgh, PA; [2]Univ. of Pittsburgh Ctr. For Res. Computing, Pittsburgh, PA; [3]Weill Cornell Grad. Sch., New York, NY; [4]Univ. of Pittsburgh Pharmacol., Pittsburgh, PA

Abstract: Prenatal exposure to synthetic glucocorticoids (sGCs) alters brain development and predisposes the developing fetus towards potential adverse neurodevelopmental outcomes. We

have used primary mouse embryonic (E14.5) neural stem and progenitor cells (NSPCs) from the telencephalon to uncover potential mechanistic bases for neurodevelopmental changes elicited by sGCs. Our previous studies demonstrated that sGCs triggered sexually dimorphic alterations in the transcriptome of embryonic mouse NSPCs. Cell-type-specific gene expression profiles tightly regulate cell fate decisions and are controlled by a flexible landscape of chromatin domains upon which transcription factors and enhancer elements act. We therefore multiplexed data from three genome-wide assays (RNA-seq, ATAC-seq (assay for transposase accessible chromatin followed by genome wide sequencing), and dual cross-linking ChIP-seq (chromatin immunoprecipitation followed by genome wide sequencing)) to identify novel relationships between gene regulation, chromatin structure, and genomic glucocorticoid receptor (GR) action in female NSPCs. These data reveal that GR binds preferentially to predetermined regions of accessible chromatin to influence gene expression that impact cell fate decisions. In addition, we identify SOX2 as a potential cell-type-enriched transcription factor that guides hormone-regulated GR action in NSPCs.

Disclosures: K. Berry: None. **U. Chandran:** None. **F. Mu:** None. **I. Rogatsky:** None. **D. DeFranco:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.01

Topic: A.07. Developmental Disorders

Support: NIH R01 MH097949

Title: Pten regulates dendritic arborization by decreasing microtubule polymerization rate

Authors: S. A. GETZ[1], K. TARIQ[2], J. R. HOWE, VI[2], P. D. SKELTON[2], J. HONG[2], ***B. LUIKART**[1];
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Abstract: Phosphatase and tensin homolog (PTEN) is a major negative regulator of the PI3K/Akt/mechanistic target of rapamycin (mTOR) pathway. Loss of function mutations in *PTEN* have been found in a subset of patients with macrocephaly and autism spectrum disorder. PTEN loss in neurons leads to somal hypertrophy, dendritic overgrowth and increased spine density, resulting in inappropriate connectivity of neuronal circuits. These neuronal overgrowth phenotypes are present upon *Pten* knockout (KO) and reconstitution with autism-associated point mutations. The mechanism underlying dendritic overgrowth in *Pten* deficient neurons is unclear. In this study, we examined how *Pten* loss impacts microtubule dynamics using retroviral

infection and transfection strategies to manipulate PTEN expression and tag plus-end microtubules. We found *Pten* KO neurons sprout more new processes compared to WT via multiphoton microscopy. We also found a significantly increased microtubule polymerization rate in *Pten* KO dendrites in primary cultured neurons via confocal microscopy. Further, reducing microtubule polymerization rate to the WT level was sufficient to reduce dendritic overgrowth in *Pten* KO neurons *in vitro* and *in vivo*. Taken together, our data suggests that one factor underlying dendritic overgrowth is increased microtubule polymerization. This opens the possibility for an intersectional approach targeting microtubule polymerization and mTOR with low doses of inhibitors to achieve therapeutic gains with minimal side-effects in pathologies associated with loss of neuronal PTEN function.

Disclosures: S.A. Getz: None. **K. Tariq:** None. **J.R. Howe:** None. **P.D. Skelton:** None. **J. Hong:** None. **B. Luikart:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.02

Topic: A.07. Developmental Disorders

Support: NSF NRT UtB:Neurophotonics Grant DGE-1633516

Title: High-resolution and automated identification of engrailed 2 transcript along spatial and cell type-specific dimensions in the ganglionic eminence of the developing mammalian brain

Authors: *J. S. MARTINEZ-FUENTES[1], M. COGSWELL[2], A. C. CASTELLÓ[4], V. BORRELL[5], S. J. RUSSEK[3];
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Abstract: A variety of genetic and environmental factors contribute to autism spectrum disorder (ASD), but exactly how this occurs remains unclear. One hypothesis is that factors may converge to perturb the proper development and function of the gamma-aminobutyric acid (GABA) system of the brain. The midbrain-hindbrain patterning homeotic transcription factor engrailed 2 (En2) has been associated with ASD in humans, and a mouse model harboring a null mutation of En2 recapitulates behavioral and neuroanatomical abnormalities reminiscent of ASD as well as epilepsy. Interestingly, En2-null mice show reduced GABAergic marker expression in the hippocampus and cortex, and cultured neural stem cells of GABAergic lineage from mutant mice display deficits in neurogenic activity. How En2 dysfunction leads to neuropathological

phenotypes, including the cell and behavioral phenotypes related to forebrain function, remains unknown. Our recent *in vivo* work draws attention to the potential role of En2 in radial glial self-renewal and subsequent differentiation, with a potential impact on the birth of GABAergic interneurons and spiny projection neurons. To aid investigation into the role of En2 in the development of forebrain GABAergic neurons, we are building a detailed transcriptomic spatial map of En2 at the mouse embryonic ganglionic eminence (GE, age E13), the major birthplace of inhibitory neurons. Here, we use sensitive multiplexed fluorescence *in situ* hybridization with widefield and confocal fluorescence microscopy to reveal spatial distributions of *En2* transcripts across transcriptionally defined subtypes of progenitor cells in regions of the GE (i.e., medial, lateral, caudal GE). To this end, we apply MATLAB automated image analysis to quantify *En2* signal in thousands of individual cells along spatial and cell type dimensions. Cell types tracked are radial glia, subapical and basal progenitors, defined by a transcriptional code of overlapping expression of GABAergic lineage factors, Gsx2 and Ascl1. Data show that *En2* is regionally widespread (~60% cells show at least one *En2* transcript), but sparse in its density (1-3 transcripts per cell) across all layers of the GE. Interestingly, in the LGE, ongoing results suggest enrichment of high-expressing *En2*+ cells in the ventral subregion, potentially indicating vulnerability of cells from this area to loss of En2 function. Taken together, widespread but sparse presence of *En2* suggests this factor may play a role in cells arising from the GE, raising questions as to how En2 dysfunction along GABAergic maturation trajectories may contribute to etiology of ASD and other developmental disorders of brain function.

Disclosures: J.S. Martinez-Fuentes: None. **M. Cogswell:** None. **A.C. Castelló:** None. **V. Borrell:** None. **S.J. Russek:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.03

Topic: A.07. Developmental Disorders

Support: NIH Grant K01NS107723

Title: Distribution of integrin β3 in the mouse brain

Authors: C. A. BRETT, C. J. HANDWERK, K. M. BLAND, H. N. COOK, B. D. SWINEHART, H. V. RODRIGUEZ, A. R. KALINOWSKI, *G. VIDAL;
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Abstract: A strong positive association has been found between mutations in integrin β3 (Itgb3) and autism spectrum disorder, but little is known about the role of integrin β3 on the structure and function of neurons and glia in vivo. Many forms of autism spectrum disorder are thought to

arise from dysfunctional dendritic arborization and dendritic spine development, and both global and brain-specific knockout of Itgb3 in mice leads to autistic-like behaviors. We have also shown that Itgb3 is required for normal dendritic arborization in layer II/III pyramidal neurons throughout the mouse neocortex and in a cell-specific manner. Prior studies suggest that integrin β3 is required in neurons of the cortex, hippocampus, and midbrain for normal brain function, and that its expression is developmentally regulated. However, the distribution of integrin β3 throughout the brain is not well characterized. We hypothesized that integrin β3 would be distributed throughout layer II/III of the cerebral cortex, the stratum pyramidale of the hippocampus, and the dorsal raphé nucleus of the midbrain. Fluorescent immunohistochemistry was used to localize integrin β3 throughout the mouse brain, and double fluorescent immunohistochemistry was used to co-label integrin β3 with various cell types. Results suggest that integrin β3 is found in multiple cell types and regions beyond the midbrain, cortex, and hippocampus. Thus, integrin β3 may serve multiple roles in the developing and adult brain.

Disclosures: C.A. Brett: None. **C.J. Handwerk:** None. **K.M. Bland:** None. **H.N. Cook:** None. **B.D. Swinehart:** None. **H.V. Rodriguez:** None. **A.R. Kalinowski:** None. **G. Vidal:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.04

Topic: A.07. Developmental Disorders

Support: Telethon-Italy GGP11043

Title: Impaired excitatory and inhibitory synaptic plasticity in the NL3-R451C mouse model of autism spectrum disorder

Authors: *M. BRUNO, S. H. STANCHEVA, L. MANNINO, M. RUBEN, A. I. POLENGHI, F. COLACI, E. M. PETRINI, A. BARBERIS;
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Abstract: Autistic spectrum disorder (ASD) has been associated to genetic alterations of proteins involved in synaptic function such as the point mutation R451C in neuroligin 3 (NLGN3), a postsynaptic adhesion molecule which binds its presynaptic partner neurexin at both excitatory and inhibitory synapses. In the present study, by exploiting the transgenic NL3R451C knock-in (KI) mice as an ASD animal model, we aimed at investigating the role of this mutation in the coordination of both excitatory and inhibitory synaptic plasticity. We found that, with respect to WT condition, the $NL3^{R451C}$ protein was less expressed at the neuronal surface and showed increased lateral diffusion at GABAergic synapses. In WT animals, we observed that, in

response to a chemical protocol for the induction of synaptic plasticity (NMDA treatment), excitatory synaptic currents were depressed (LTD) whereas the inhibitory ones were potentiated (iLTP). Interestingly, such opposed synaptic plasticity was abolished in KI neurons. These effects were paralleled by changes in synaptic receptors and scaffold proteins at both excitatory and inhibitory synapses in WT and KI neurons. Indeed, after the NMDA stimulation, WT mice showed increased immunoreactivity for GABAAR and the scaffold protein gephyrin at synapses. In contrast, no significant changes were observed in KI mice. Along the same line, NMDA treatment induced the decrease of the GluA1 and GluA2 subunits of AMPA receptors in WT mice, whereas the same protocol left AMPARs unchanged in KI mice. In addition, the quantification of NLGN3 clusters fluorescence intensity revealed that, after the NMDA treatment, surface NLGN3 decreased in WT neurons while it was unaffected in KI neurons. Moreover, we demonstrate that in KI, changes in the expression of the NR2B subunit of NMDA receptor were associated with altered intracellular spatio-temporal calcium dynamics. Collectively, our results reveal that the perturbed synaptic molecular composition of both glutamatergic and GABAergic synapses and the disrupted calcium signaling induced by the NL3 R451C mutation disrupts the coordination of excitatory and inhibitory synaptic plasticity thus potentially contributing to the pathophysiology of ASD.

Disclosures: M. Bruno: None. **S.H. Stancheva:** None. **L. Mannino:** None. **M. Ruben:** None. **A.I. Polenghi:** None. **F. Colaci:** None. **E.M. Petrini:** None. **A. Barberis:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.05

Topic: A.07. Developmental Disorders

Support: UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Rose Hills Foundation Graduate Scholarship Training Program
NIH Grant R01DA051897

Title: A reduction in the autism associated gene lysine demethylase 6B (KDM6B) blocks neuronal differentiation and alters microglia activity.

Authors: *J. BUTH[1], J. EMTAGE[2], F. TURCIOS[2], A. DURRANI[2], T. ISHIDA[2], B. NOVITCH[3];
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Abstract: Autism spectrum disorder (ASD) is a lifelong developmental disability that affects how an individual experiences and interacts with the world. Individuals experience challenges with social interaction, communication, and repetitive behaviors. Current treatments include behavioral therapy, medications, and assistive technology focused on improving the patient's quality of life, but the underlying cause remains unclear. Current studies point to deficits in synapse formation/pruning, altered excitatory/inhibitory neuron balance, increased microglial activation, and astrocytosis. Many ASD risk genes, including lysine demethylase 6B (KDM6B), regulate gene expression through chemical modification of histone and chromatin proteins. Emerging evidence suggests that KDM6B is involved in balancing microglial activation states as well as regulating neural differentiation. Microglia are known to regulate immune responses, brain homeostasis, neuronal differentiation, and synapse maintenance. KDM6B knockdown in mice is associated with a reduction in cortical neurons, but it is unclear if this is due to deficits in neuron formation, overactive microglia, or both. As many clinical trials for neurological disorders based on mouse data fail, there is a need to validate hypotheses and therapeutics with human models. In this study, we aimed to distinguish the effect of KDM6B loss in hPSC-derived NPCs and microglia (**Fig. 1**). To model a reduction in KDM6B activity, we used a highly specific inhibitor of KDM6B demethylase activity (GSK-J4) and an inert isomer (GSKJ5) as an additional control. We infected a 2D monolayer of NPCs with a lenti-Syn1-RFP virus to visualize the production of neurons. Addition of GSK-J4 resulted in fewer RFP+ neurons (**Fig 2**) indicating a reduction in neurogenesis. For microglia we assayed gene expression, proliferation, phagocytosis, and migration. Addition of GSK-J4 altered the expression of activation markers, proliferation, phagocytosis, and increased migration toward ADP (**Fig. 3-6**). These results suggest pathological changes seen in ASD due to KDM6B loss may arise from changes in NPCs as well as microglia. Understanding these cell specific effects helps narrow the possible therapeutic targets and predict possible outcomes. Future studies will focus on how KDM6B loss affects cell-cell interactions using 3D brain organoids integrated with microglia. These experiments could provide a better understanding of how ASD arises and could expand treatments to further improve quality of life.

Disclosures: J. Buth: None. **J. Emtage:** None. **F. Turcios:** None. **A. Durrani:** None. **T. Ishida:** None. **B. Novitch:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.06

Topic: A.07. Developmental Disorders

Support: National Research Foundation Grant Number 118524

Title: Implications for Autism Spectrum Disorders: mitochondrial dysfunction and neuronal differentiation in SH-SY5Y cells

Authors: E. BUCHANAN, E. FRICKEL, C. MAHONY, S. BAM, *C. O'RYAN;
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Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with numerous genes and biological pathways implicated in its aetiology. We previously identified differentially methylated genes enriched for canonical mitochondrial pathways in a South African ASD cohort. Mitochondrial dysfunction in this cohort was supported by metabolomic analysis. We therefore hypothesise that aberrant expression of key mitochondrial genes, such as PCCB, contributes to ASD pathogenesis via altered neurogenesis resulting from mitochondrial dysfunction. PCCB deficiency leads to an accumulation of propionate, which is used in a rat model to study ASD. We aimed to recapitulate PCCB-related mitochondrial dysfunction using sodium propionate (NaP) treatment (n=3) compared to controls (n=3) in SH-SY5Y neuronal-like cells and characterise the downstream effects on neuronal differentiation. NaP treatment significantly dysregulated mitochondrial biogenesis (1.5mM NaP/24hr), reduced mtDNA copy number (qPCR of ND1 relative to RPPH1) and upregulated PGC-1α expression (3mM NaP/24hr). In addition, NaP also resulted in mitochondrial membrane depolarisation as measured using mitotracker fluorescent staining. Changes in mitochondrial morphology (fusion and fission) were observed using cryogenic transmission electron microscopy and fluorescence microscopy, where mitochondrial hyperfusion was seen at lower NaP concentrations and mitochondrial fragmentation at higher NaP concentrations. These morphological changes were supported by altered expression of mitochondrial fusion (STOML2, MFN2) and fission (DPR1) genes. Significance was determined using a one way ANOVA ($P<0.05$). Finally, NaP-induced mitochondrial dysfunction resulted in temporal and morphological changes during neurodifferentiation, characterised by immunocytochemistry and fluorescence microscopy. Our data show that NaP treatment alters mitochondrial function and dynamics and dysregulates differentiation in SH-SY5Y cells. These findings offer insight into the potential molecular mechanisms underlying mitochondrial dysfunction and suggest a mechanism of how this may contribute to ASD aetiopathogenesis via altered neurogenesis.

Disclosures: E. Buchanan: None. **E. Frickel:** None. **C. Mahony:** None. **S. Bam:** None. **C. O'Ryan:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.07

Topic: A.07. Developmental Disorders

Support: R01MH112714
R01MH114858
1R21MH100868
1R01NS08916
Nancy Lurie Marks Family Foundation
Landreth Foundation
Autism Speaks/National Alliance for Autism Research

Title: Increasing UBE3A in substance P (Tac1) neurons of VMHvl hypothalamic nucleus increases aggression-associated neuronal activity

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Abstract: Heightened aggression is a common comorbidity of autism spectrum disorder (ASD) including excessive tantrums, irritability, and self-injurious aggressive behaviors that often requires medical treatment. Yet the molecular and neuronal circuit mechanisms underlying the ASD-associated aggression and treatment response remains undefined. We previously reported that transgenic mice with full-length *Ube3a* (*Ube3a2x* mice, modeling ASD due to maternal *15q11-13* triplication), compared with wild type littermates, display increased total attack time and attack numbers in the resident intruder task. We further found that targeted increases of *Ube3a* in the *Tac1*- or *Pgr*-expressing neurons of ventromedial hypothalamus, ventrolateral subdivision (VMHvl) increases aggression. Yet, the activity of VMHvl *Tac1* neurons during aggression has not been examined. In the current study, we used head-mounted nVista miniature microscopy (Inscopix) to image intracellular calcium dynamics (a neural activity correlate) in VMHvl *Tac1* neurons when mouse conducting aggression behavior. Activity of multiple VMHvl *Tac1* neurons with or without increased *Ube3a* were monitored in freely behaving animals to correlate *Tac1* neuron activity with aggression behavior. *AAV-hSyn-DIO-Gcamp7* or *AAV-hSyn-DIO-Gcamp7 + AAV-hSyn-DIO-Ube3a* virus were injected into VMHvl of *Tac1-Cre* male mice, and follow by implanting ProView Lens Probes, baseplate installation later. We performed a resident-intruder aggression test paradigm as follow: 5 min control followed by introducing female intruder for 5 mins, and then after removing the female, introducing a male intruder to measure the aggression behavior. Calcium images of the VMHvl *Tac1* neurons were acquired using Inscopix Data Acquisition Software during the above 20 min test paradigm. VMHvl *Tac1* neurons showed low basal activity when resident mice were alone in their home-cages. Introducing a female intruder enhanced activity in a limited subset of cells. However, introducing a male intruder dramatically increased activity of all VMHvl *Tac1* neurons identified. By summing the amplitude of calcium spike values during the 5 minute epochs during baseline, female exposure, and male exposure, we found that VMHvl *Tac1* neuron activity is increased upon male intruder addition with additional bursts of activity correlated to attack behaviors. Increasing UBE3A in these VMHvl *Tac1* neurons further elevated this neuronal activity. The results indicate VMHvl *Tac1* neuron activity strongly correlates to aggression behavior and this activity is further magnified by increased UBE3A.

Disclosures: Y. Nong: None. **M. Anderson:** None.

Poster

P046. Autism: Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P046.08

Topic: A.07. Developmental Disorders

Support: NIH R01MH110487-06
PHRF

Title: The psychiatric risk gene Transcription Factor 4 (TCF4) regulates the density and connectivity of a specific subclass of inhibitory interneurons.

Authors: *H.-Y. CHEN[1], B. PHAN[2], S. SRIPATHY[1], G. R. HAMERSKY[1], J. BOHLEN[1], G. SHIM[1], T. O'DONNELL[1], A. R. PFENNING[3], B. J. MAHER[4];
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Abstract: Transcription Factor 4 (TCF4) is a type I basic helix-loop-helix transcription factor and autosomal mutations in TCF4 causes Pitt-Hopkins Syndrome (PTHS), a neurodevelopmental disorder with autistic features. The pathophysiology resulting from TCF4 haploinsufficiency or expression of a dominant negative TCF4 protein remains incomplete. During embryonic development, GABAergic interneurons originate from the proliferative zones of the ventral forebrain and in situ hybridization suggests Tcf4 transcript is enriched in this brain region. In addition, single cell RNAseq data indicates Tcf4 is highly expressed across many subclasses of GABAergic interneurons. Moreover, TCF4 is a known dimerization partner of ASCL1 (or MASH1), a critical regulator of interneuron development. Therefore, we hypothesized that TCF4 may play a critical role in regulating interneuron development and function. To test this idea, we quantified GABAergic interneuron density in a PTHS mouse model that harbors a truncated Tcf4 gene (Tcf4+/tr) and compared it to WT littermates. Immunohistochemistry (IHC) for the major cortical GABAergic markers showed a significant decrease in the PV+ population across several brain areas including medial prefrontal cortex, primary motor cortex, striatum, and basal lateral amygdala. Likewise, qPCR and cell-type specific expression analysis (CSEA) of bulk RNAseq data also predicted a significant decrease in the proportion of PV+ cells. Interestingly, this deficit was specific to PV+ cells, as IHC and CSEA analysis of SST+ interneurons was not different. Furthermore, we quantified interneuron density by crossing a tdTomato reporter line with a variety of GABAergic subclass-specific cre lines and observed that Tcf4 mutation resulted in a reduction in PV+ interneurons across all brain regions measured, while VIP+ cells showed a significant reduction only in the motor cortex and SST+ interneuron density was unchanged. Lastly, we characterized the impact Tcf4 mutation had on the physiology of PV+ interneurons by

performing targeted whole-cell electrophysiology. We observed no differences in the intrinsic membrane properties, however the frequency of spontaneous inhibitory synaptic currents was significantly reduced. Together, these results suggest that the density and connectivity of inhibitory neurons in the PTHS mouse is perturbed, and these changes may in part underlie the cognitive deficits and autistic features present within this patient population.

Disclosures: H. Chen: None. **B. Phan:** None. **S. Sripathy:** None. **G.R. Hamersky:** None. **J. Bohlen:** None. **G. Shim:** None. **T. O'Donnell:** None. **A.R. Pfenning:** None. **B.J. Maher:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.01

Topic: B.04. Ion Channels

Support: NIH grant DC01919
Autifony Therapeutics Ltd.

Title: Kv3.4 channels bind the cell adhesion molecule PCDH9 to regulate axonal pathfinding

Authors: *Y. ZHANG[1], M. CHATTERJEE[1], N. DELUCA[1], J. WU[1], M. MARABITA[2], A. BIOSA[2], N. PILATI[2], M. J. GUNTHORPE[2], C. H. LARGE[2], L. K. KACZMAREK[1]; [1]Yale Univ. Sch. Med., New Haven, CT; [2]Autifony Therapeut. Limited, Stevenage, United Kingdom

Abstract: The voltage-dependent potassium channel Kv3.4 is present in the growth cones of developing neurons, and its presence is required for accurate axonal pathfinding and elongation in embryonic neurons. The specific role of the channel in these processes is, however, unknown. We carried out a yeast two-hybrid screen of a rat brain library using the cytoplasmic C-terminal domain of Kv3.4 as bait and found that protocadherin 9 (PCDH9) was the most represented interacting protein. PCDH9 is a calcium-dependent cell-cell adhesion molecule that, like Kv3.4, is found in the growth cones of developing fibers. We confirmed this interaction by co-immunoprecipitation in both Kv3.4 expressing cell line and mouse brain tissue. To examine whether there is a functional interaction between the two proteins, we used Crispr Cas9 to knock down PCDH9 from Kv3.4 expressing cells. We found that loss of PCDH9 resulted in near total suppression of both Kv3.4 currents and Kv3.4 protein expression. Kv3.4 currents and protein levels were recovered on overexpression of human PCDH9 into the PCDH9-knockout cells. We used cultures of cerebellar granule cells, which express high levels of Kv3.4, to assess the effects of knockdown or overexpression of this channel subunit. Loss of Kv3.4 reduced axonal branching, synapse formation, and retarded the normal developmental pattern of neurite extension. Conversely, overexpression of Kv3.4 in the neurons accelerated the maturation of

neuronal morphology. Our results strongly suggest that normal function of the Kv3.4 channel requires its binding to PCDH9 and that this channel/adhesion molecule complex regulates axonal pathfinding.

Disclosures: Y. Zhang: None. **M. Chatterjee:** None. **N. DeLuca:** None. **J. Wu:** None. **M. Marabita:** None. **A. Biosa:** None. **N. Pilati:** None. **M.J. Gunthorpe:** None. **C.H. Large:** None. **L.K. Kaczmarek:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.02

Topic: B.04. Ion Channels

Support: NIH grant DC01919
Autifony Therapeutics Ltd.

Title: Role of voltage gated potassium channel Kv3.4 in neuronal development and neurite outgrowth

Authors: *M. CHATTERJEE[1], Y. ZHANG[1], J. WU[1], M. MARABITA[2], A. BIOSA[2], N. PILATI[2], M. GUNTHORPE[2], C. LARGE[2], L. K. KACZMAREK[1];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Autifony Therapeut. Limited, Stevenage, United Kingdom

Abstract: Role of voltage gated potassium channel Kv3.4 in neuronal development and neurite outgrowth
M. CHATTERJEE[1], Y. ZHANG[1], J. WU[1], M. MARABITA[2], A. BIOSA[2], N. PILATI[2], M. J. GUNTHORPE[2], C.H. LARGE[2], L. K. KACZMAREK[1];[1]Yale Univ. Sch. Med., New Haven, CT, 06510, USA and [2]Autifony Therapeutics Limited, Stevenage Bioscience Catalyst, Stevenage SG1 2FX, UK
Voltage gated Kv3.4 channels are abundantly expressed in cerebellum, hypothalamus and cerebellar cortex amongst other brain regions. These channels are involved in guidance and pathfinding of neurons during early embryonic development in mice. In this study, we explored modulation of Kv3.4 expression and its impact on the neurite extension, complexity and maturation with particular emphasis on cerebellar granule neurons in culture. In addition, we explored the effects of pharmacological modulators and blockers of Kv3.4 channel on the degree of neurite extension and the complexity of dendritic arbors. We observed that knocking down Kv3.4 expression using CRIPSR-Cas9 significantly retarded the development of cerebellar granule neurons whereas overexpressing the channels by using a plasmid expressing Kv3.4 accelerated their rate of development compared to control cells. Tetraethylammonium (TEA), a

potassium channel blocker also retarded the development of the cerebellar granule neurons, and had effects similar to those of knockdown of Kv3.4 expression. Overall, the results suggest that Kv3.4 channels play an important role in the morphological development and maturation of CG neurons.
This work was supported by NIH grant DC01919 to LKK and in part by Autifony Therapeutics Ltd.

Disclosures: M. Chatterjee: None. **Y. Zhang:** None. **J. Wu:** None. **M. Marabita:** None. **A. Biosa:** None. **N. pilati:** None. **M. Gunthorpe:** None. **C. Large:** None. **L.K. Kaczmarek:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.03

Topic: B.04. Ion Channels

Support: NIH Grant NS116327
Georgia State University Brains and Behavior Pre-doctoral Fellowship

Title: Investigating KChAP, a voltage-dependent K^+(Kv) channel interacting protein, as a putative E3 small ubiquitin-like modifier (SUMO) ligase for the Kv4 ternary complex

Authors: *L.-A. R. JANSEN[1], M. A. WELCH[1], D. J. BARO[1,2];
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Abstract: Early studies on Kv channel interacting proteins described a novel protein, Kv Channel Associated Protein (KChAP) that interacted with Kv1 and Kv2 alpha-subunits and Kvbeta-subunits in yeast two hybrid assays, and co-immunoprecipitated (co-IP) with Kv2.1 and Kv4.3 in native heart tissue. In heterologous expression systems, KChAP co-expression enhanced the currents mediated by Kv1.3, Kv2.1, and Kv4.3, significantly decreased the currents mediated by Kv1.2 and Kv1.4, but had no effect on Kv1.5. KChAP was discovered to be identical to Protein Inhibitor of Activated STAT 3 (PIAS3). At the time, PIAS3 was thought to act solely as a transcription factor regulator, but surprisingly, transcription was not necessary for KChAP effects on the Kv4 current, IA. That is where the story ended around 2002. Subsequently, unrelated studies determined that PIAS3 was an E3 ligase for SUMO peptides, which are covalently attached to lysine residues on target proteins to modulate target protein interactions. Thus far, no link has been made between KChAP and ion channel SUMOylation. We are investigating if KChAP acts as a SUMO ligase for the Kv4 family of ion channels. Kv4 exists in a ternary complex with K^+ channel interacting proteins (KChIP1-4) and the dipeptidyl peptidase like-proteins (DPP6,10). Preliminary data show that co-expression of KChAP with KChIP2a, DPP10 and either Kv4.1, Kv4.2 or Kv4.3 increased IA relative to the expression of

ternary complex components alone. SUMOylation of Kv4.2 at K579 produced a significant 36% increase in the maximal conductance (Gmax) of IA mediated by the Kv4.2 ternary complex by reducing channel internalization (see Welch et al poster). To determine if KChAP increased the Kv4.2 ternary complex IA by SUMOylating Kv4.2 K579, we co-expressed KChIP2a and DPP10 with a SUMOylation deficient Kv4.2, Kv4.2(K579R), with and without KChAP. KChAP produced a significant increase in IA mediated by the Kv4.2(K579R) ternary complex relative to controls lacking KChAP ($p = 0.0281$, t-test); however, it should be noted that KChAP produced a significant 88% increase in IA mediated by the wild type Kv4.2 ternary complex ($p=0.004$, t-test), but only a 33% increase in IA mediated by the mutant Kv4.2(K579R) ternary complex; thus, removing a known SUMO site on Kv4.2 significantly diminished the effect of KChAP. Together, the data suggest that KChAP may be SUMOylating multiple sites on the ternary complex. Additional SUMO sites are predicted for Kv4.2, and KChIP2a and DPP10 contain high probability SUMO sites. We are performing IP experiments followed by western blots to determine if members of the ternary complex are SUMOylated by KChAP.

Disclosures: L.R. Jansen: None. **M.A. Welch:** None. **D.J. Baro:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.04

Topic: B.04. Ion Channels

Support: NIH intramural funding

Title: A p38-Pin1-Kv4.2 cascade regulates hippocampal excitability and memory processing

Authors: *C. MALLOY[1], J. HU[2], J. J. GUTZMANN[3], A. PRATT[2], M. AHERN[2], D. A. HOFFMAN[4];
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Abstract: The A-type potassium current (I_A) is a vital regulator of neuronal excitability. In rodent hippocampal CA1 pyramidal cells, this current is carried primarily by voltage-gated Kv4.2 K+ channels. Along with other voltage-gated ion channels localized in the somatodendritic compartment of pyramidal cells, Kv4.2 contributes to the firing mode of the cell. Kv4.2 functions in complex with the auxiliary subunits K+ channel interacting proteins (KChIPs) and DPLP(s) to regulate dendritic excitability and synaptic plasticity in the mouse hippocampus. Proper assembly and dynamic regulation of this complex is integral in facilitating it's role in neuronal signal processing and impairments in this process can manifest in disease. To illuminate potential molecular modifiers of the complex, we used a proteomic screen. We

identified a molecular cascade involving p38 kinase-mediated Pin1 isomerization of a C-terminal motif in Kv4.2 as a crucial regulator of Kv4.2-DPP6 binding dynamics. To probe the role of this p38-Pin1 cascade in regulating Kv4.2 function, Crispr-cas9 technology was utilized to generate a mouse model (Kv4.2TA) with abolished p38 and Pin1-Kv4.2 binding. We confirmed disruption of endogenous Kv4.2-Pin1 binding by co-immunoprecipitation from whole brain lysates. We investigated the physiological consequence of this reduced binding on neuronal and network excitability using hippocampal whole-cell patch clamp electrophysiology and behavioral seizure tests in adult mice. Kv4.2TA mice displayed reduced action potential (AP) firing frequency in response to stepped somatic current injections. This reduction in neuronal excitability was replicated by pharmacological blockade of Pin1 and p38 activity in wild type (WT) mice. To confirm a direct influence by p38-Pin1 on Kv4.2-mediated I_A, we performed voltage-clamp recordings from outside-out somatic patches pulled from CA1 pyramidal cells. Isolation of I_A revealed a significant increase in current density in patches pulled from Kv4.2TA cells relative to WT. This increase in somatic I_A and reduced neuronal excitability was accompanied by a reduction in kainic-acid induced seizure intensity. Lastly, Kv4.2TA mice displayed enhanced reversal learning in multiple tests, indicating an improvement in cognitive flexibility. Measurements of synaptic plasticity in the hippocampus of Kv4.2TA and WT mice are on-going in order to ascribe a cellular mechanism to this phenotype. We, therefore, have illuminated a novel molecular cascade that is an important regulator of hippocampal excitability and memory processing.

Disclosures: C. Malloy: None. **J. Hu:** None. **J.J. Gutzmann:** None. **A. Pratt:** None. **M. Ahern:** None. **D.A. Hoffman:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.05

Topic: B.04. Ion Channels

Title: Mechanisms of maladaptive compensation in KCNQ2-related epilepsy in patient-specific iPSC-derived neurons

Authors: *D. SIMKIN[1], M. GHARIB[1], A. L. GEORGE, JR[2], E. KISKINIS[1];
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Abstract: Heterozygous loss-of-function mutations in *KCNQ2*, which encodes a voltage-gated K^+ channel subunit responsible for neuronal M-current, have been associated with neonatal developmental and epileptic encephalopathy (KCNQ2-DEE). This complex disorder manifests as severe early-onset seizures and impaired neurodevelopment due to an imbalance in neuronal

circuit activity in the brain. While the effects of *KCNQ2* mutations have been studied extensively in heterologous expression systems, their effects on the inherent properties of human neurons are poorly understood. Specifically, what remains unclear is how defects in M-current affect the electrophysiological properties of human neurons during a critical neurodevelopmental maturation period. In our recent published study (PMID 33544076), we demonstrated that iPSC-derived neurons from a patient with KCNQ2-DEE exhibit enhanced tonic burst-suppression-like firing on multi-electrode arrays (MEAs). In contrast to isogenic mutation-corrected controls, KCNQ2-DEE neuron bursting becomes progressively phasic as neurons mature. This maladaptive maturation time course is associated with upregulation of Ca^{2+}-activated K^+ channels leading to faster action potential (AP) repolarization and larger post-burst after-hyperpolarizations (AHP) over several weeks in culture. While this maladaptive homeostatic response could be replicated in control neurons by chronically inhibiting M-channels pharmacologically, it is not clear whether induction of this homeostatic response is specifically due to altered M-channel function or altered neuronal activity that results from M-channel inhibition.
Here we build upon these recent findings and use KCNQ2-DEE patient-specific and isogenic mutation-corrected control iPSC-derived excitatory neurons from multiple patients as well as unrelated healthy controls to investigate the mechanisms involved in eliciting this maladaptive homeostatic response. We find that chronic treatment of control neurons with M-channel blocker XE991 leads to an aberrant bursting phenotype while blocking other voltage-gated K^+ channels with nonspecific blocker 4AP does not. We hypothesize that upregulation of Ca^{2+}-activated K^+ channels associated with this bursting phenotype is likely a specific response to M-channel dysfunction. Because KCNQ2-DEE presents in the first days of life, targeting the underlying cause (i.e. KCNQ2) may be challenging, and identifying novel downstream targets may enable alternative therapeutic strategies.

Disclosures: D. Simkin: None. **M. Gharib:** None. **A.L. George, Jr:** None. **E. Kiskinis:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.06

Topic: B.04. Ion Channels

Support: NSF Grant 2030348

Title: Differences between the kcnb1-integrin machinery in normal and disease states

Authors: *A. BORTOLAMI[1], W. YU[1], E. FORZISI[1], R. KADAKIA[1], F. SESTI[2];
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Abstract: Potassium channels regulate neuronal excitability in the central nervous system. This regulationallows information processing, which eventually results in a motor response. Furthermore,neuronal excitability regulation is not the only process mediated by potassium channels; theyare also capable of eliciting biochemical events. Over the past decade, our laboratory has beenstudying a voltage-gated potassium ion channel, KCNB1, which not only explicates the canonicion-conduction function but is also implicated in cell migration through intracellular signaling. Ina previous study, we characterized the association of the KCNB1 channel with integrins,essential intramembrane proteins involved in the outside-in cell signaling. Furthermore,integrins are not the only proteins associated with this channel. Through biochemical analysis,we started to characterize the intracellular machinery associated with both KCNB1 andintegrins, which are responsible for the channel-associated biochemical signaling cascades.Evidences highlights paxillin, talin-1, and vinculin as main candidates for this role. Ourpreliminary work showed that mutations in KCNB1 found in children with epilepsy can lead tocytoskeleton remodeling impairments. Accordingly, we found that those mutants impair talin-1and paxillin activities. Thus, non-ionic functions of Kcnb1 channels proceed through integrinsand their associated machinery and these are impaired in disease conditions.

Disclosures: A. Bortolami: None. **W. Yu:** None. **E. forzisi:** None. **R. Kadakia:** None. **F. Sesti:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.07

Topic: B.04. Ion Channels

Support: NIH GM007324
NIH DC01919
NIH NS18492

Title: Pathogenic R455H mutation of Slack (KCNT1) channels causes misregulation of β-actin translation initiation

Authors: *T. MALONE[1], Y. ZHANG[3], J. WU[3], R. CHEN[2], P. LICZNERSKI[4], E. A. JONAS[3], **L. K. KACZMAREK**[3];
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Abstract: The Slack channel is a large conductance sodium-activated potassium channel expressed in the cerebral cortex and many other neuronal nuclei. Over 50 gain-of-function Slack mutations are associated with early-onset epilepsy and severe intellectual disability in humans.

One unexplained aspect of Slack pathogenesis is the source of intellectual disability in these patients. Misregulation of mRNA translation is a possible explanation for this phenotype, because Fragile X Mental Retardation Protein (FMRP), an important regulator of mRNA translation, binds the Slack protein directly and also binds Slack mRNA. The binding of FMRP to Slack stimulates its channel activity, raising the possibility that activation of Slack channels could also regulate the function of FMRP. To test this hypothesis, we used a fluorescent reporter construct with its coding region flanked by the 5' and 3' UTR of β-actin, a known FMRP binding target, and detected levels of reporter using immunoblotting and measurements of fluorescence. Both total protein and translation levels of the reporter construct were markedly increased in cells expressing the R474H Slack mutant but not the wild type control, an effect mediated by the β-actin UTRs. FRAP experiments used to visualize real-time protein synthesis found that pharmacological activation of WT Slack channels triggers increased synthesis of the reporter protein. This mechanism of activity-dependent translation is inhibited by both FMRP and its binding partner CYFIP1. We verified that this mechanism is common to both rodent and human Slack channels and that it operates in mouse cortical neurons. Current experiments are testing the mechanism by which channel activity is linked to translation and its implications for neuronal function. These findings potentially represent the first example of the direct modulation of protein synthesis by activation of an ion channel and suggest that abnormal regulation of translation may explain the intellectual disability of patients with Slack mutations.

Disclosures: T. Malone: None. **Y. Zhang:** None. **J. Wu:** None. **R. Chen:** None. **P. Licznerski:** None. **E.A. Jonas:** None. **L.K. Kaczmarek:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.08

Topic: B.04. Ion Channels

Support: SFARI Program of the Simons Foundation
NIH R01 MH104450

Title: Autism spectrum disorder-associated voltage-gated potassium channel mutant Kv7.3/R230C disrupts mouse dopamine neuron activity.

Authors: *C. TSCHUMI, B. JUAREZ, M. A. QUINLAN, J. H. HAYANO, L. S. ZWEIFEL; Univ. of Washington, Seattle, WA

Abstract: Neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD), have complex etiologies and a diversity of phenotypic outcomes. A growing body of evidence implicates deficits in mesostriatal circuitry as contributing to several aspects of behavioral

dysregulation associated with these disorders. Underlying these circuit-level dysfunctions, alterations in the brain-wide function of specific ion channels is emerging as a key factor in the etiology of NDDs.
The Kv7 family of voltage-gated potassium channels encoded by KCNQ genes are increasingly linked to NDDs including ASD. These channels are broadly expressed, including within ventral tegmental area (VTA) dopamine neurons where they regulate neuronal excitability. Voltage-gated ion channels, including Kv7s, are gated by the movement of positively charged arginine (R) residues in the S4 transmembrane domain. Mutations in all three arginine (R) residues that constitute the gating charges of the voltage sensor in the Kv7.3 subunit (KCNQ3) have been identified in NDDs including ASD. The KCNQ3(R230C) mutation exhibits an increase in potassium conductance consistent with a gain of function mutation. However, it remains unclear how gating charge mutations in KCNQ3 alter neuronal excitability.
Here we assessed the impact of KCNQ3(R230C) on VTA dopamine neuron excitability using a viral-based strategy for conditional inactivation of endogenous Kv7.3 by CRISPR/SaCas9 coupled with conditional re-expression of human wildtype (hKv7.3/WT) or mutant (hKv7.3/R2C) Kv7.3 in mice. CRISPR/Cas-9 inactivation of the mouse Kcnq3 gene alone did not alter the proportion of spontaneously active cells, but substantially increased the frequency of spontaneous and evoked action potential firing. Expression of hKv7.3/WT rescued these effects. In striking contrast, hKv7.3/R2C expression almost completely prevented spontaneous activity, decreased firing in response to depolarizing current injection, increased the spike frequency accommodation during trains of action potential firing, and broadened the action potential waveform. The opposing effects of Kv7.3 inactivation with CRISPR/Cas9 and re-introduction of hKv7.3/R2C on excitability are consistent with a gain of function in the hKv7.3/R2C channel and not a loss of function. These data demonstrate that the R230C mutation in hKV7.3 alters the action potential waveform and excitability of neurons in vivo that likely disrupts the function of multiple neural networks, including the mesostriatal dopamine system, that contribute to behavioral dysregulation in ASD.

Disclosures: C. Tschumi: None. **B. Juarez:** None. **M.A. Quinlan:** None. **J.H. Hayano:** None. **L.S. Zweifel:** None.

Poster

P096. Potassium Channels: Physiological Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P096.09

Topic: B.04. Ion Channels

Support: NIH Grant NS102239

Title: Mechanisms of KCNT1 channel gain-of-function in epilepsy and intellectual disability

Authors: *J. WU, Y. ZHANG, I. QURAISHI, M. CHATTERJEE, L. K. KACZMAREK; Yale Univ. Sch. Med., New Haven, CT

Abstract: Gain-of-function (GOF) mutations in the *KCNT1* gene, encoding the sodium-activated potassium channel KCNT1 (Slack, $K_{Na}1.1$), are associated with several distinct childhood epilepsies, all of which are associated with very severe developmental intellectual disability (ID). The underlying mechanisms of KCNT1 channel GOF in epilepsy and ID are, however, largely unknown. We have generated a mutant strain of mice with human *KCNT1* variant *KCNT1*-R474H (R455H in mouse numbering) and investigated the effect of *KCNT1*-R455H GOF variant on neural network excitability leading to seizure generation. We classified primary cortical neurons as glutamatergic and GABAergic and carried out electrophysiological recordings on the two types of neurons from wild type and R455H mutation mice. Voltage-clamp studies revealed that *KCNT1* variant R455H increased peak K_{Na} current magnitude in both excitatory glutamatergic and inhibitory GABAergic neurons. Current-clamp studies revealed that this mutation significantly increases the rate of action potential (AP) repolarization in glutamatergic neurons, leading to an increase in AP firing frequency. In contrast, the R455H mutation increases rheobase and decreases input resistance in GABAergic neurons, leading to a reduction in AP firing frequency. These finding suggest that excitatory or inhibitory neurons differ in either the KCNT1 splice variants they express, or in the spatial proximity of KNCT1 channels to sites of sodium influx. Overall, these results suggest that the *KCNT1*-R455H GOF variant leads to neural network hyperexcitability and produce early onset seizures by altering the intrinsic excitability of neurons. Seizures arise from increased excitation in excitatory neurons and reduced excitability in inhibitory interneurons. Our study may lead to novel methods and targets of treating epilepsy and neurodevelopmental disorders in patients with *KCNT1* GOF mutations.

Disclosures: J. Wu: None. **Y. Zhang:** None. **I. Quraishi:** None. **M. Chatterjee:** None. **L.K. Kaczmarek:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.01

Topic: B.11. Glial Mechanisms

Support: This work was supported in part by: Cure Alzheimer's Fund

Title: Dissection of artificial and confounding glial signatures by single cell sequencing of mouse and human brain

Authors: *S. E. MARSH[1,2,3], A. J. WALKER[1,2,3], T. KAMATH[3,2], L. DISSING-OLESEN[1,2,4], T. R. HAMMOND[1,2,3], T. DE SOYSA[1,2,3], A. M. H. YOUNG[5], S. MURPHY[1], A.

ABDULRAOUF[3], N. NADAF[3], C. DUFORT[1], A. C. WALKER[1], L. E. LUCCA[6], V. KOZAREVA[3], C. VANDERBURG[3], S. HONG[7], H. BULSTRODE[5], P. J. HUTCHINSON[8], D. J. GAFFNEY[9], D. A. HAFLER[6,4], R. FRANKLIN[5], E. Z. MACOSKO[3,10], B. STEVENS[1,2,3,11]; [1]F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Stanley Ctr. for Psychiatric Res., [4]Broad Inst. of MIT and Harvard, Cambridge, MA; [5]Wellcome-Medical Res. Council Cambridge Stem Cell Institute, Cambridge Biomed. Campus, Univ. of Cambridge, Cambridge, United Kingdom; [6]Dept. of Neurol. and Dept. of Immunobiology, Yale Sch. of Med., New Haven, CT; [7]UK Dementia Res. Inst., Univ. Col. London, London, United Kingdom; [8]Dept. of Clin. Neurosciences, Univ. of Cambridge and Cambridge Univ. Hosp. NHS Fndn. Trust, Cambridge, United Kingdom; [9]Wellcome Genome Campus, Hinxton, Wellcome Sanger Inst., Cambridgeshire, United Kingdom; [10]Depart of Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [11]Boston Children’s Hosp., Howard Hughes Med. Inst., Boston, MA

Abstract: A key aspect of nearly all single-cell sequencing experiments is dissociation of intact tissues into single-cell suspensions. While many protocols have been optimized for optimal cell yield, they have often overlooked the effects that dissociation can have on *ex vivo* gene expression. Generation of new and reanalysis of published mouse single-cell RNA-sequencing (scRNA-seq) datasets demonstrates that use of enzymatic dissociation on mouse brain tissue induces an aberrant *ex vivo* gene expression signature, most prominently in microglia, that can significantly confound downstream analyses.

To minimize this issue in fresh tissue, we developed and validated a flexible protocol that maintains cell type diversity and yield, while preserving *in vivo* transcriptional profiles. Reanalysis of previously published mouse and human scRNA-seq of multiple tissue types reveals that many aspects of this signature are conserved across tissues/organs and species. We were further able to identify a similar signature in post-mortem human brain in newly-generated and published single-nucleus RNA-sequencing datasets, and show that this signature is induced in freshly-isolated human brain tissue and peripheral immune cells by exposure to elevated temperatures *ex vivo*.

Our results of fresh tissue dissociation provide key insight into the potential confounds of enzymatic digestion and provide a solution to allow for enzymatic digestion for scRNA-seq while avoiding *ex vivo* transcriptional artifacts in both mouse and human tissue. The results of our analysis of post-mortem and fresh human brain samples indicates that the presence of a similar signature in astrocytes and microglia may be the result of a combination of acute pre-mortem and post-mortem variables, which combined may confound the identification and interpretation of true biological signals. Together our results provide a methodological solution for preventing artifactual gene expression changes during fresh tissue digestion and a reference for future deeper analysis of the potential confounding states present in post-mortem human samples.

Disclosures: S.E. Marsh: None. **A.J. Walker:** None. **T. Kamath:** None. **L. Dissing-Olesen:** None. **T.R. Hammond:** None. **T. de Soysa:** None. **A.M.H. Young:** None. **S. Murphy:** None. **A. Abdulraouf:** None. **N. Nadaf:** None. **C. Dufort:** None. **A.C. Walker:** None. **L.E. Lucca:** None. **V. Kozareva:** None. **C. Vanderburg:** None. **S. Hong:** None. **H. Bulstrode:**

None. **P.J. Hutchinson:** None. **D.J. Gaffney:** None. **D.A. Hafler:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dr. Hafler has received research funding from Bristol-Myers Squibb, Sanofi, and Genentech for work unrelated to this project. F. Consulting Fees (e.g., advisory boards); Dr. Hafler has consulted for Bristol Myers Squibb, Compass Therapeutics, EMD Serono, Genentech, Juno therapeutics, Novartis Pharmaceuticals, Proclara Biosciences, Sage Therapeutics, & Sanofi Genzyme. **R. Franklin:** None. **E.Z. Macosko:** None. **B. Stevens:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS R01NS081040
UM SIP 2019-2
F31NS115225
e Miami Project to Cure Paralysis, the Buoniconti Fund to Cure Paralysis

Title: Microglia heterogeneity in the injured CNS using single-cell RNAseq.

Authors: *J. CHOI[1], L. M. MILICH[1], J. K. LEE[2];
[2]The Miami Project to Cure Paralysis, [1]Univ. of Miami, Miami, FL

Abstract: Microglia are a critical component in the inflammatory milieu after a CNS injury - they perform diverse functions such as produce cytokines, clear cellular debris, and initiate repair. While recent studies have used single-cell RNAseq approaches to study these heterogeneous roles, most have been under homeostasis or in neurodegenerative disease. Whether microglia execute unique or conserved responses to both traumatic and degenerative disease is not well known. To address this, we performed an integrated single-cell RNAseq analysis of published datasets in neurodegeneration and traumatic injury. We observed both unique and conversed transcriptional programs across diseases and attempted to elucidate determinants of microglia fate using trajectory analysis. This comparative analysis may provide insight into the mechanisms by which microglia respond to injuries and reveal potential therapeutic targets.

Disclosures: J. Choi: None. **L.M. Milich:** None. **J.K. Lee:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.03

Topic: B.11. Glial Mechanisms

Support: NIH Grant EY030307

Title: Distinct microglial recognition pathways mediate apoptotic cell clearance versus remodeling transcriptome and CSF1R independence

Authors: *S. ANDERSON[1], J. M. ROBERTS[1], N. GHENA[1], A. BOSCO[2], E. IRVIN[1], J. SCHWAKOPF[1], I. COOPERSTEIN[1], M. L. VETTER[3];
[1]Univ. of Utah, Salt Lake City, UT; [2]Dept Neurobiol Anat, Univ. of Utah, Salt Lake Cty, UT; [3]Dept Neurobiol & Anat., Univ. Utah Sch. Med., Salt Lake Cty, UT

Abstract: Microglia are essential for developmental remodeling of the nervous system and recent single-cell RNA sequencing (scRNAseq) has uncovered substantial transcriptional diversity of microglia during brain development. We previously found that a majority of microglia (≈60%) in the postnatal mouse retina have a gene signature similar to disease-associated microglia (DAM) and microglia in developing white matter tracts (PAM/ATM), and that they do not depend upon Colony-stimulating factor 1 receptor (CSF1R) signaling for survival. Here we sought to address how developmental cues and specific signaling pathways promote these distinct microglia properties. We performed scRNAseq on postnatal (P6/P7) retinal microglia from both males and females (n=13,667 cells), and found a spectrum of homeostatic to more DAM/PAM/ATM-like states. Preventing neuronal apoptosis via loss of BAX (n=13 animals), shifted the microglia population to a more homeostatic state. Conversely, CSF1R resistant microglia (PLX3397 dosed for 3 days; n≥11 animals) were enriched for phagocytosis and lipid metabolism genes and more DAM/PAM/ATM-like microglia. Thus, recognition or clearance of apoptotic neurons drives microglia to express DAM/PAM/ATM genes and reduces dependence on CSF1R signaling. To define the signaling pathways regulating these microglia properties, we analyzed a panel of apoptotic recognition receptor knockouts. We found that complement receptor CD11b/CR3, and Mertk of the TAM family, are each required for the clearance of apoptotic retinal ganglion cells (n≥4; p=0.006 and p<0.001). The TAM receptor Axl was less important for clearance (n=8, ns), but was required for microglial independence from CSF1R (n=17; p<0.001). We further showed that loss of both Mertk and Axl shifts microglia to a more homeostatic state.
To assess whether DAM/PAM/ATM-like microglia have specialized clearance function, we showed using CD11c-DTR/GFP transgenic mice that the majority of CD11c-microglia interact with apoptotic cells (n=5; ~73%), and depleting these cells (DT) reduces clearance (n≥7; p<0.05). However, we found that depleting non-CD11c-microglia (PLX3397) similarly reduces clearance (n=10; n<0.001), suggesting CD11c-microglia may not be specialized to do so.Thus, microglial heterogeneity within the developing retina is driven by recognition and phagocytosis of apoptotic neurons. Further, Mertk and CR3 are important for apoptotic neuron clearance, Axl

is required for CSF1R independence, and CD11c-microglia arise in response to exposure to apoptotic neurons, but do not have an enhanced clearance function.

Disclosures: S. Anderson: None. **J.M. Roberts:** None. **N. Ghena:** None. **A. Bosco:** None. **E. Irvin:** None. **J. Schwakopf:** None. **I. Cooperstein:** None. **M.L. Vetter:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.04

Topic: B.11. Glial Mechanisms

Support: NINDS, R01NS112144
NINDS, R01NS088627

Title: Cns-associated macrophages do not repopulate from microglia

Authors: *L. WANG[1,2], J. ZHENG[1,2], T. CHEN[3], S. ZHAO[3], L.-J. WU[2,3,4];
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Abstract: Microglia and CNS-associated macrophages (CAMs) are long-live immune cells in the CNS, which can be ablated by the drug PLX3397 and fully repopulate after PLX3397 retraction. Given that both microglia and CAMs originate from the yolk sac progenitors, and that microglia are solely repopulated from the residual microglia during recovery from ablation, we wanted to investigate whether the CAMs could be replenished by microglia during repopulation. We used Tmem119-CreER:Rosa26$^{TdT/+}$ transgenic mice to genetically trace and distinguish microglia from CAMs, where microglia were Iba1^{+}TdTomato^{+} while CAMs were Iba1^{+}TdTomato^{-}. We first checked the label efficiency and specificity of this Cre-flox system, and the results showed that approximately 90% of microglia (Cd206^{-}Iba1^{+}) were labeled with TdTomato (cortex microglia 94%±5.57, hippocampus microglia 88.67%±5.77) while none of the CAMs (Cd206^{+}Iba1^{+}) are TdTomato positive. Mice were fed with the PLX3397 chow for 14 days, which successfully ablated approximately 98% of Iba1^{+} cells in the brain, and 99% of the Cd206^{+}Iba1^{+} CAMs in both the meninges and the perivascular space. PLX3397 chow was retracted from day 15, and the normal chow was supplied to let microglia and CAMs repopulate. We collected brain tissue at day 3, day 7 and day 14 of the repopulation period and examined the microglia and CAM populations by immunofluorescent staining. The results showed that no CAMs (Cd206^{+}Iba1^{+}) repopulated in the meninges, perivascular space or choroid plexus were TdTomato positive at all these timepoints. Previous studies showed that microglia with the mutation of the transmembrane protein *negative regulator of reactive oxygen species* (NRROS) expressed more CAM marker protein including Cd206, indicating the interchangeability between

microglia and CAMs under certain circumstances. Thus, it's reasonable to hypothesize that the microglia could replenish the CAMs during repopulation. However, our study demonstrated that the CNS-associated macrophages do not repopulate from microglia, further revealing the subtle differences between the microglia and CAMs. This may suggest the different roles of microglia and CAMs in various pathological conditions including encephalitis and cerebrovascular diseases.

Disclosures: L. Wang: None. **J. Zheng:** None. **T. Chen:** None. **S. Zhao:** None. **L. Wu:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.06

Topic: B.11. Glial Mechanisms

Support: NIH NRSA F32 1F32MH118785
SFARI 491371

Title: Neuro-immune organoid model reveals a role of microglia in cell stress response and emergence of neural activity

Authors: *G. POPOVA, S. S. SOLIMAN, C. N. KIM, M. G. KEEFE, K. M. HENNICK, D. SHIN, T. J. NOWAKOWSKI;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Many fundamental questions about microglia function during brain development in humans remain unanswered as we lack experimental strategies to interrogate their function and responses *ex vivo*. Xenotransplantation of microglia into brain organoids that develop largely without myeloid lineage cells allows a systematic comparison of neurodevelopmental processes in the presence or absence of microglia. By developing a 'neuro-immune' cerebral organoid model, we systematically characterized 3D brain organoids transplanted with primary human microglia and showed that microglia integrate into the organoids and affect brain development. By performing scRNAseq analysis between transplanted and control organoids, we detected subtle but statistically significant differences at transcriptional level in a cell type-dependent manner. Unexpectedly, we found widespread expression of genes involved in type I interferon response in organoid radial glia, which becomes attenuated in the presence of microglia. In contrast, neurons appear transcriptionally invariant to microglia transplantation, but instead undergo synaptic remodeling and an accelerated maturation of network properties. Functional network activity recordings in brain organoids identified a role for human microglia in modulating synaptic density and accelerating the emergence of synchronized oscillatory network activity, similarly to what has been reported in maturing cortical organoids. In agreement with

the role of microglia in synaptic pruning, we detected synaptic material inside of the microglia cells and overall reduction of synaptic clefts in microglia-transplanted organoids. Together, our work reveals transcriptional and functional responses of the developing human neural tissue to the presence of microglia and establishes a novel model for studying neuro-immune interactions and developmental diseases in a context of human tissue.

Disclosures: G. Popova: None. **S.S. Soliman:** None. **C.N. Kim:** None. **M.G. Keefe:** None. **K.M. Hennick:** None. **D. Shin:** None. **T.J. Nowakowski:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIGMS 5P20GM103427
Haddix Grant Creighton University

Title: Regulation of Microglial Anti-Viral Response by Long Non-Coding RNAs

Authors: O. BURLEIGH[1], N. W. MATHY[2], K. DRESCHER[2], X.-M. CHEN[3], *A. SHIBATA[1]; [1]Biol., [2]Sch. of Med., Creighton Univ., Omaha, NE; [3]Rush Univ., Chicago, IL

Abstract: Multiple Sclerosis (MS) is the most common demyelinating disease of the central nervous system. The exact mechanisms and cause of this disease are still largely unknown; however, one leading hypothesis is that the disease is triggered by a viral infection. Theiler's murine encephalomyelitis virus (TMEV) is a single-stranded RNA cardiovirus and is commonly used to model chronic progressive MS. Long non-coding RNAs (lncRNAs) are a type of RNA that are not transcribed into protein and are at least 200 nucleotides long. LncRNAs modulate diverse cellular processes and play a key role in inflammation. The molecular mechanisms of lncRNAs in inflammatory neurodegenerative disorders such as MS are currently unknown. We hypothesized that after TMEV infection in microglia, certain lncRNAs are differentially expressed and alter the viral load in infected microglia. To test our hypothesis in vitro, we infected a murine microglial cell line (BV2) and primary murine microglia with the Daniels (DA) strain of TMEV at 5 MOI for 4 hours. Following infection, cells were rinsed with PBS, and fresh media was added. Viral infection proceeded for 24 hours before RNA was isolated for RT-qPCR analysis. To investigate TMEV infection in vivo, 4–6-week-old FBV/nJ mice were intracerebrally injected with TMEV, or Hank's balanced salt solution (HBSS) in control mice. Animals were sacrificed after 36 days during the chronic demyelination stage and cortical tissue was collected. Functional studies using knockdown or overexpression of lncRNAs were performed in BV2 cells and primary murine microglial cells using a liposomal transfection

technique. GAPDH was used as an endogenous control in RT-qPCR analysis and relative expression was calculated using the comparative Ct (ΔΔCt) method. TMEV infection of BV2s, primary microglia, and FVB/nJ mice significantly upregulated expression of lncRNA-Nostrill compared to controls. UV-inactivated TMEV failed to induce lncRNA-Nostrill in in vitro experiments. Knockdown of lncRNA-Nostrill significantly increased TMEV viral load in BV2 cells while overexpression of lncRNA-Nostrill significantly reduced TMEV viral load. A 24-hour TMEV infection in primary microglia significantly upregulated pro-inflammatory cytokines IL-1ß and TNF-α by 3.2-fold and 5.2-fold, respectively. Interestingly, there was also an upregulation of the anti-inflammatory cytokine IL-10 by 10.3-fold. Further work will investigate the underlying molecular mechanisms of lncRNA-Nostrill, its contribution to anti-viral responses in the central nervous system, and its potential role in the amelioration of demyelination in a virus-induced mouse model of MS.

Disclosures: O. Burleigh: None. **N.W. Mathy:** None. **K. Drescher:** None. **X. Chen:** None. **A. Shibata:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PRIN17

Title: Purinergic signaling in microglia and potential role of neurosteroids in current modulation

Authors: *M. BARRACO[1], E. KUDOVA[2], M. A. SORTINO[1], M. CHISARI[1];
[1]Dept. of Biomed. and Biotechnological Sciences, Section of Pharmacol., Univ. of Catania, Catania, Italy; [2]Inst. of Organic Chem. and Biochem., Czech Acad. of Sci., Prague, Czech Republic

Abstract: Neuroinflammation is a hallmark of neurodegenerative diseases. It involves elevation of ATP levels and several types of glial cells, including microglia. Microglial cells express several purinergic ionotropic receptors, P2XRs, and among them P2X4 and P2X7 receptor subtypes seem to be involved in anti- or pro-inflammatory processes, respectively. Moreover, cholesterol metabolites, including oxysterols and neurosteroids, may modulate neuroinflammatory responses. In this study we tested the hypothesis that cholesterol metabolites modulate purinergic current in the BV2 microglia cell line, using patch-clamp techniques. We first isolated current elicited by P2X4 and P2X7 receptor subtypes using different ATP concentrations (5 μM and 300 μM, respectively). Then, selective antagonists for each receptor subtype were used to verify P2X4 and P2X7 current. For P2X4 receptors, 5-BDBD (10 μM)

reduced current by 70%, whereas A438079 (10 μM) nearly full inhibited P2X7 current. Afterwards, cells were exposed to cholesterol metabolites (testosterone butyrate/T-BT 20 μM, 17β-estradiol 1 μM, Δ4-dafachronic acid/DAF 10 μM and allopregnanolone/alloP 10 μM) to evaluate changes in current. To estimate P2X4 receptor modulation, cells were exposed to ATP to elicit basal current and then exposed to cholesterol metabolites for 30 s. Basal P2X4 peak amplitude (normalized to 1) was enhanced by T-BT (1.7 ± 0.15, $n = 8$, $p < 0.001$), DAF (2.46 ± 0.14, $n = 7$, $p < 0.0001$) and alloP (1.26 ± 0.07, $n = 12$, $p < 0.01$), but not by 17β-estradiol (1.09 ± 0.05, $n = 14$, $p > 0.05$). P2X7 current in BV2 cells show a strong increase with repeated ATP application. For this reason, we set a control condition where the second ATP exposure was normalized to the first one, and in a different set of experiments, we applied cholesterol metabolites for 30 s prior the second ATP application. We then normalized the increase in current to the first ATP application and we compared both normalized ATP second stimulations to evaluate any current modulation by cholesterol metabolites. P2X7 peak amplitude was not affected by any cholesterol metabolites used in this study. However, T-BT application strongly increased P2X7 receptor rise time (ATP 3324 ms ± 220.1 vs T-BT 707 ms ± 76.1, $n = 10$, $p < 0.0001$). Taken together, these data suggest that cholesterol metabolites modulate P2X4 current more ubiquitously than P2X7. P2X4 current increase elicited by several compounds and the faster rise time for P2X7 receptor by T-BT might influence intracellular pathways in microglia, suggesting new approaches to treat neuroinflammation.

Disclosures: M. Barraco: None. **E. Kudova:** None. **M.A. Sortino:** None. **M. Chisari:** None.

Poster

P161. Microglia: Biology II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P161.08

Topic: B.11. Glial Mechanisms

Support: R01NS088627
R01NS112144

Title: Microglial interaction with neurovasculature in the maintenance of blood-brain barrier in acute seizures

Authors: *K. HARUWAKA, Y. LI, L.-J. WU;
Mayo Clin., Rochester, MN

Abstract: The maintenance of the blood-brain barrier (BBB) is required for homeostasis of the brain environment. Previous studies suggest that microglia, the resident immune cells in the central nervous system, contribute to BBB protection by the direct interaction with vascular endothelial cells during systemic inflammation (Haruwaka et al., 2019). However, the role of

microglia and their interaction with neurovasculature in BBB integrity in neurological disorders is largely unknown.Recent studies demonstrated that BBB is compromised in seizures and epilepsy. As well as BBB breakdown may induce epileptic seizures (Vilet et al., 2007; Dadas and Janigro, 2019), seizure-induced BBB leakage may lead to further progression of epilepsy (Rüber et al., 2018; Mendes et al., 2019). The recovery of BBB dysfunction after seizures may improve prognosis. In addition, microglial depletion aggravates the severity of kainic acid (KA) and pilocarpine-induced acute and chronic seizures (Mirrione et al., 2010; Wu et al., 2020). Those results suggest a beneficial role of microglia in suppressing seizures. We hypothesize that microglia-vasculature interaction may have a key role BBB permeability in the pathogenesis of seizure. To this end, we investigate the role of microglia and their interaction with neurovasculature during KA-induced mouse model of seizures.We first study the time-course changes in BBB leakage in KA-induced severe seizures. After both KA intracerebroventricular (ICV) and intraperitoneal (IP) injection of KA, we found that 70 kDa dextran transiently leaked peaking 6 hours after the induction of the seizure. In addition, the percentage of vessel-associated microglia dramatically increased after 1 day and 3 days. To further delineate the role of microglia in seizure-induced BBB leakage, we used microglial ablation approaches. We found that KA-induced BBB leakage was dramatically increased in mice genetically and pharmacologically depleted of microglia. These results suggest that the protective function of microglia in BBB permeability in acute seizures.Defining microglia-vasculature interaction in BBB integrity represents an important step toward understanding the microglial function in epilepsy and may provide potential therapeutic targets for neuroprotection in compromised BBB in neurological disorders.

Disclosures: K. Haruwaka: None. **Y. Li:** None. **L. Wu:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P51-OD011107-57S3
NIH Grant P51- OD011107-58S2

Title: Tau seeding and propagation in a novel monkey model of Alzheimer's pathogenesis.

Authors: *D. BECKMAN[1], P. CHAKRABARTY[3], S. OTT[1], A. DAO[1], E. ZHOU[1], W. JANSEN[4], K. K. COX[1], S. MULLER[5], J. H. KORDOWER[5], J. MORRISON[1,2];
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Abstract: Alzheimer's disease (AD) is a devastating condition that affects around 5.8 million of Americans, and these numbers are projected to reach nearly 14 million individuals around 2050. Although AD was first described more than 100 years ago, until nowadays that are not effective treatments to counteract or efficiently slow down the progression of the disease. This is likely, due at least in part, to the fact that AD animal models have been focused in the use of transgenic rodent models, failing to translate the findings to humans with the disease. We describe here a non-human primate model of AD presenting extensive tau pathology, mainly affecting the hippocampus and connected areas. Adult rhesus monkeys injected with adeno-associated viruses expressing mutant tau (P301L/S320F) in the left entorhinal cortex (ERC) show after 3 months, extensive prion-like tau propagation with full fibrillary tangles in the hippocampus, as well as pre-tangles and phospho-tau in projection areas such as the contralateral ERC and in the retrosplenial and visual cortices. Importantly, neuroinflammation is extensive in the affected areas, with a major role of microglia in pathological tau oligomer propagation. Finally, the mutant h4R injected coaptates monkey 3R tau generating more tau aggregation and spreading. These results highlight the first stages of tau pathology and propagation and support the importance of a non-human model of AD, with natural full expression of tau protein, that is highly translational to humans.

Disclosures: D. Beckman: None. **P. Chakrabarty:** None. **S. Ott:** None. **A. Dao:** None. **E. Zhou:** None. **W. Jansen:** None. **K.K. Cox:** None. **S. Muller:** None. **J.H. Kordower:** None. **J. Morrison:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Office of the Director award P51-OD011107

Title: Transsynaptic spread combined with glia-driven neuroinflammatory response orchestrate the neuro-invasive potential of SARS-CoV-2 in an aged monkey model of COVID-19

Authors: *A. BONILLAS[1], D. BECKMAN[1], G. B. DINIZ[1], S. OTT[1], K. K. A. VAN ROMPAY[1], S. S. IYER[2], B. A. SCHMIDT[2], J. MORRISON[1,3];
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Abstract: The COVID-19 pandemic continues to affect millions of people globally, with unpredictable levels of morbidity and mortality. Recent reports state that more than 80% of patients with COVID-19 develop neurological symptoms that can vary from loss of smell,

hemorrhagic stroke, to psychiatric symptoms. Even for those who initially recover, the majority still present cognitive issues, among other neurological problems, many months after the initial infection. Considering that COVID-19 has a severe impact on the aging population, the long-term social and economic burden of the current pandemic can reach alarming levels. To understand how SARS-Cov-2 virus can affect the brain, we developed a rhesus monkey model of COVID-19 pathogenesis. In this pilot study, 4 young healthy (6-9 Y.O) and 4 aged diabetic (21-22 Y.O) rhesus monkeys were infected intranasally and intratracheally with SARS-CoV-2 at a high dose (2.5x106 plaque-forming units [PFU]) and euthanized 7 days later, capturing the peak T cell plasma response to investigate the neuro-invasive potential of the virus. Additional age-matched non-infected animals were analyzed as controls. Our initial unbiased stereological analysis shows the direct presence of several SARS-CoV-2 markers such as spike protein, nucleocapsid and double-stranded RNA (dsRNA) across several cortical regions, with significantly more viral presence and spreading in the aged diabetic animals. Olfactory regions such as the piriform, orbitofrontal, and entorhinal cortices present severe blood-brain barrier (BBB) disruption, with several myeloperoxidase (MPO) activated neutrophils, SARS-CoV-2+ infiltrating the surrounding areas only 7 days after infection. Astrocytic population doubled in the piriform of aged diabetic monkeys in comparison to the young infected ones, suggesting an accelerated mechanism of inflammatory response driven by the active presence of the virus in that likely entered through the olfactory pathway. Strikingly, activated HLA-Dr+ microglia directly attack large pyramidal neurons. SARS-CoV-2+ in the primary olfactory cortex result in robust engulfment of spine proteins such as PSD95 and synaptophysin, ultimately leading to neuronal death. Our preliminary data helps shed light on the neuro-invasive and spreading potential of SARS-CoV-2 across several brain regions in a monkey model of the disease. Identifying the underlying molecular mechanisms of the viral propagation in the primate brain will offer a unique possibility to apply the rhesus monkey model as a platform to test therapies to counteract SARS-CoV-2 spreading, and ultimately, reducing the burden of COVID-19 long-term complications.

Disclosures: **A. Bonillas:** A. Employment/Salary (full or part-time):; California National Primate Research Center. **D. Beckman:** A. Employment/Salary (full or part-time):; California National Primate Research Center. **G.B. Diniz:** A. Employment/Salary (full or part-time):; California National Primate Research Center. **S. Ott:** A. Employment/Salary (full or part-time):; California National Primate Research Center. **K.K.A. Van Rompay:** A. Employment/Salary (full or part-time):; California National Primate Research Center. **S.S. Iyer:** A. Employment/Salary (full or part-time):; UC Davis. **B.A. Schmidt:** A. Employment/Salary (full or part-time):; UC Davis. **J. Morrison:** A. Employment/Salary (full or part-time):; California National Primate Research Center.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P20 GM103429
Arkansas Academy of Science Undergraduate Research Grant
Trey Carlock Rising Scholar Award

Title: Microglia polarize in response to transactive response DNA-binding protein-43 (TDP-43) and display partial recovery after removal of the stimulus

Authors: *A. WILCOX, D. DONLEY;
Harding Univ., Searcy, AR

Abstract: Transactive response DNA binding protein-43 (TDP-43) is an intranuclear protein but mislocalization is associated with several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal lobe dementia (FTD). TDP-43 is released into the extracellular space where it is sensed by microglia, the CNS-resident immune cells. Our data and the literature suggest that microglia respond to TDP-43 dysregulation by increasing CNS inflammation. The goal of this study was to determine the impact of extracellular TDP-43 on microglial function and the extent to which microglia recover after stimulation. To study the inflammatory response, cultured immortalized microglia were stimulated with TDP-43 in a 2x2 factorial design with other inflammatory stimuli. Data were analyzed by ANOVA. Using iNOS and Arginase colorimetric assays, we found that TDP-43 caused microglia to lose the ability to appropriately respond to inflammation. Stimulation with TDP-43 increased iNOS activity in response to interferon-gamma and dampened Arginase activity in response to IL-4/IL-13. These results are evidence that TDP-43 polarizes microglia to a pro-inflammatory state, thus altering the response to cytokines and other inflammatory stimuli. A natural function of microglia is engulfment of neuronal synapses. We found that extracellular TDP-43 increased microglial phagocytosis of neurons in culture. Previous research showed improvements in cognitive function when TDP-43 was removed by antibody treatment, which suggests that microglia may be able to recover after removal of the stimulus. To study recovery, cultured microglia were stimulated with TDP-43 or a vehicle; then the media was changed to remove the stimuli and allow a recovery period. Markers of activation were measured using flow cytometry and metabolic assays. After recovery, microglia had a decreased phagocytic capability as compared to TDP-43 stimulation without recovery. Microglia also demonstrated a metabolic shift toward glycolysis in response to TDP-43, consistent with a proinflammatory phenotype but returned to baseline levels of metabolic activity after recovery. These data demonstrate that dysregulated TDP-43 shifts the balance of signaling pathways toward an inflammatory phenotype, but microglia begin to recover after removal of the inflammatory stimuli. More work is needed to understand the mechanisms that control recovery and the phenotypic shifts that occur in response to extracellular stimuli. Future work will also focus on the extent to which these finding contribute to disease progression in neurological diseases such as ALS and FTD.

Disclosures: A. Wilcox: None. **D. Donley:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: P01NS074969
AG061776

Title: How does sleep impact neuroinflammation in Alzheimer's disease?

Authors: *S. PARHIZKAR[1], G. MORTIMER[1], N. RENSING[1], M. GRATUZE[1], J. ROMERO[1], G. STROUT[2], D. TIMM[1], C. WANG[1], C. LEE[1], J. REMOLINA SERRANO[1], X. BAO[1], M. MANIS[1], M. FINN[1], J. D. ULRICH[1], R. TOWNSEND[3], S. SVIBEN[2], J. FITZPATRICK[2], A. CASHIKAR[1], M. WONG[1], D. M. HOLTZMAN[1];
[1]Neurol., Washington Univ. Sch. of Med., St. Louis, MO; [2]Washington Univ. Ctr. for Cell. Imaging, Washington Univ. Sch. of Med., Saint Louis, MO; [3]Med., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Sleep loss is associated with cognitive decline in older adults, and is likely a risk factor for Alzheimer's disease (AD), the most common cause of dementia. However, the underlying mechanisms are unclear. Recently our lab highlighted that sleep loss promotes deposition of the key pathological hallmarks of AD, Aβ and tau aggregates, in mouse models of AD. Considering the crucial role of immunomodulating genes such as the triggering receptor expressed on myeloid cells type 2 (TREM2) in clearing pathogenic Aβ aggregates, our aim was to investigate whether the sleep/wake cycle influences neuroinflammation, another key player in AD pathogenesis. Using amyloid-depositing 5xFAD mice expressing humanized TREM2 ($T2^{cv}$), we show that sleep deprivation (SD) for six hours per day over six weeks, starting at the age of plaque onset, significantly increased disease-associated microglia markers such as IBA1, Clec7a and CD68, as well as GFAP+ astroglia compared to age- and genotype-matched normally sleeping (NS) controls. Consequently, P2RY12, a marker of homeostatic microglia, decreased upon SD compared to NS 5xFAD/$T2^{cv}$ controls. This suggests that microglia and astroglia, the key immune-regulating cells in the brain, directly respond to sleep loss. We also observed elevated pro-inflammatory cytokine levels of TNFα and ILβ upon SD in brain homogenates from SD compared to NS 5xFAD/$T2^{cv}$ mice. Moreover, SD further increased fibrillar Aβ load compared to age-matched NS 5xFAD/$T2^{cv}$ mice, which may explain the elevated microglial and astroglial reactivity. However, we show similar effects in SD wild-type mice compared to NS mice, suggesting that sleep loss promotes a proinflammatory environment in the brain in the presence or absence of Aβ pathology. Additionally, we found a TREM2-dependent change in sleep/wake behavior where 5xFAD/$T2^{cv}$ mice spent more time awake in the dark phase

compared to age-matched 5xFAD mice expressing the AD-associated R47H TREM2 variant ($T2^{R47H}$) or lacking endogenous TREM2 ($T2^{KO}$). In line with these findings, the increased microglial and astroglial reactivity observed in SD 5xFAD/$T2^{cv}$ mice did not significantly change in SD 5xFAD/$T2^{R47H}$ or 5xFAD/T2KO controls, indicating a TREM2-dependent effect on neuroinflammation driven by changes in sleep. In conclusion, our findings suggest that sleep loss acts as an early pathological stressor that promotes a neuroinflammatory cascade, which may further exacerbate AD pathology, eventually leading to neurodegeneration and dementia.

Disclosures: S. Parhizkar: None. **G. Mortimer:** None. **N. Rensing:** None. **M. Gratuze:** None. **J. Romero:** None. **G. Strout:** None. **D. Timm:** None. **C. Wang:** None. **C. Lee:** None. **J. Remolina Serrano:** None. **X. Bao:** None. **M. Manis:** None. **M. Finn:** None. **J.D. Ulrich:** None. **R. Townsend:** None. **S. Sviben:** None. **J. Fitzpatrick:** None. **A. Cashikar:** None. **M. Wong:** None. **D.M. Holtzman:** F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Takeda, Casma, Eli Lilly.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 13161291
University of Virginia Brain Institute
We acknowledge the Keck Center for Cellular Imaging for the use of the Leica SP5X microscopy system (PI_AP; NIH-RR025616)

Title: Macrophage interactions with axonal spheroids inhibit neurodegeneration

Authors: *S. HUNTER-CHANG[1,2], Y. YONG[2], T. VEGIRAJU[2], S. KUCENAS[2], C. DEPPMANN[1,2];
[1]Univ. of Virginia Neurosci. Program, Charlottesville, VA; [2]Biol., Univ. of Virginia Biol., Charlottesville, VA

Abstract: Axon degeneration is a key process in most pathological neurodegeneration and in all developmental neurodegeneration. As they degenerate, axons pass through an initial “latent” phase in which normal functions continue and gross morphology is unchanged, then through a “catastrophic degeneration” phase in which the axon rapidly disintegrates. This transition from salvageable quiescence to irreversible destruction might be explained by the bubble-like “axonal spheroids” that form on degenerating axons. Our previous work found that spheroid rupture releases axonal contents into extracellular space, and that these contents hasten degeneration of intact axons exposed to them. Additionally, we have observed that spheroids are marked with a

phosphatidylserine (PS) “eat me” signal known to induce engulfment by macrophages. However, whether macrophages selectively target spheroids for engulfment and how this engulfment affects the switch from quiescence to catastrophic degeneration remains unknown. To address this, sympathetic neurons were cultured in microfluidics devices with or without endogenous GFP-labeled macrophages, then the neurons were injured by enucleation. Neurodegeneration 0-4 hours post-injury was quantified as percent neuronal signal loss using confocal microscopy. Compared to uninjured conditions, neurons injured with macrophages present showed no significant increase in degeneration, while those injured without macrophages present did (n=3 uninjured, n=5 injured). Ongoing work seeks to validate these findings *in vivo* in zebrafish, *Danio rerio*, and to specify if and how macrophages preferentially contact spheroids. This work demonstrates that interactions between macrophages and axonal spheroids inhibit neurodegeneration, elucidating a new role for macrophage infiltration and debris clearance in response to neuronal injury and highlighting new targets for neurodegeneration therapies.

Disclosures: S. Hunter-Chang: None. **Y. Yong:** None. **T. Vegiraju:** None. **S. Kucenas:** None. **C. Deppmann:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.06

Topic:

Support: NIH/NIA R01AG069433
R01AG060718
R56063405

Title: Trem2-induced activation of microglia contributes to synaptic resilience in non-demented individuals with Alzheimer’s neuropathology

Authors: *A. FRACASSI[1], M. MARCATTI[1], R. WOLTJER[2], S. MORENO[3], G. TAGLIALATELA[1];
[1]Neurol., Univ. of Texas Med. Br., Galveston, TX; [2]Oregon Hlth. & Sci. Univ., Portland, OR; [3]Univ. Roma Tre, Rome, Italy

Abstract: The existence of individuals who remain cognitively intact despite the presence of histopathological signs of Alzheimer’s disease (AD), here referred to as “Non demented with AD neuropathology” (NDAN), suggests that some unknown mechanisms are triggered to resist cognitive impairment[1]. Synaptic dysfunction has been identified as one of the major AD causes and it is established that microglia, attracted to plaques, phagocyte damaged synapses[2]. In this context, exposed phosphatidylserine (ePS) represents a neuronal “eat-me” signal involved in

microglial-mediated phagocytosis of damaged synapses[3]. A possible candidate mediator of this process is represented by TREM2, a microglial surface receptor activated by ligands including PS. Based on TREM2 role in the scavenging function of microglia, we hypothesize that an efficient microglial phagocytosis of damaged synapses underlies synaptic resilience in NDAN, thus protecting from memory deficits. Using immunofluorescence microscopy, a comparative study of human *post-mortem* frontal cortices of aged-matched individuals, AD and NDAN individuals has been performed. The distribution of activated microglia (IBA1 and IBA1/CD68 positive cells) and the expression of phagocytic microglia-related proteins (TREM2 and DAP12) were evaluated in relation to Aβ plaques. To test the efficacy of microglia in removing debris and damaged synapses, preservation of synapses around plaques was assessed using MAP2 and tubulin βIII as dendritic and axonal markers respectively. As a further confirmation of microglial efficiency, we looked for engulfed damaged synapses inside microglial lysosomes analyzing the colocalization between PSD95 and CD68. Immunofluorescence analyses indicate higher microglial activation and TREM2 expression in NDAN individuals, as well as preserved axonal and dendritic structure around plaques *vs*. AD. High levels of PSD95 around NDAN plaques and the colocalization of PSD95 and CD68 may suggest a prompt removal of damaged synapses by hyperactive phagocytic microglia. Furthermore, taking advantage of flow cytometry techniques, a study of the ePS using Annexin V assay has been performed. Annexin V assay on synaptosomes isolated from aged-matched, AD and NDAN individuals indicates changes in the ePS in NDAN individuals, confirming the engulfment of damaged synapses. Our results suggest a higher efficiency of TREM2-induced phagocytic microglia in removing damaged synapses, underlying synaptic resilience in NDAN individuals. 1. Zolochevska and Taglialatela, 2016. 2. Edwards 2019. 3. Scott-Hewitt et al., 2020.

Disclosures: A. Fracassi: None. **M. Marcatti:** None. **R. Woltjer:** None. **G. Taglialatela:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MICINN/MIU (FPU16/04259 to EPM; PID2019-106943RB-I00 to EW)
JCyL (SA129P20 to EW)
USAL

Title: Oleoylethanolamide attenuates neuroinflammation and microgliosis induced by the cerebellar neurodegeneration of the PCD mutant mouse

Authors: *E. PÉREZ-MARTÍN[1,2], L. PÉREZ-REVUELTA[1,2], P. TÉLLEZ DE MENESES[1,2], D. DÍAZ LÓPEZ[1,2], E. WERUAGA[1,2];
[1]INCYL, Inst. for Neurosci. of Castile and Leon, Univ. of Salamanca, Salamanca, Spain;
[2]IBSAL, Inst. of Biomed. Res. of Salamanca, Salamanca, Spain

Abstract: The endocannabinoid oleoylethanolamide (OEA) has been proven to exert anti-inflammatory and neuroprotective effects in different animal models with brain injury of varied etiology. Indeed, a previous study of our laboratory demonstrated that the exogenous administration of OEA -prior to the onset of the neurodegenerative process- resulted in neuronal protection of Purkinje cells and improvement of the behavioral defects observed in *Purkinje Cell Degeneration* (PCD) mutant mice. In this study, we tested whether OEA treatment (10 mg/kg, i.p. at postnatal day 12) modulates neuroinflammation and attenuates microglial activation in the mouse model of severe cerebellar degeneration PCD. First, changes in mRNA levels of different proinflammatory, neurotrophic and neuroprotective factors in the cerebellum were measured by quantitative PCR in the short- (3- and 24-hours post-treatment), medium- (P20) and long-term (P30; *n*=6 animals per group). Secondly, modulation of microglial activation and its phenotype was analyzed in parasagittal sections of cerebellar vermis by immunohistochemistry at P30 when the neurodegenerative process has ended (*n*=7 animals per group). Our results showed that OEA treatment significantly reduced mRNA levels of the proinflammatory factors IFNγ, IL1β and TNFα, and increased the neurotrophic/neuroprotective factors BDNF, GAP43 and MAP2 in cerebellum 3 and 24 hours after administration. Additionally, treated animals showed an increase in the mRNA levels of all the analyzed factors in the medium term comparing with untreated animals. By contrast, a decrease in mRNA levels of IL1β and IL6 was observed in those treated animals in the long term. Finally, results from immunohistological analysis of cerebellar microglial cells showed that OEA treatment reduced the microglial density in the cerebellum of PCD mice, which was exacerbated as a consequence of neurodegeneration. These findings demonstrate the neuroimmunomodulatory properties of OEA in the PCD mouse and shed light on the possible mechanisms of action of OEA as a therapeutic agent which could be a co-occurrence among down-regulation of neuroinflammation, modulation of microglial activation and neuronal protection.

Disclosures: E. Pérez-Martín: None. **L. Pérez-Revuelta:** None. **P. Téllez de Meneses:** None. **D. Díaz López:** None. **E. Weruaga:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Institut Pasteur de Montevideo - FOCEM Mercosur (COF 03/11)
Amyotrophic Lateral Sclerosis Association (00482)
Agencia Nacional de Investigación e Innovación (ANII)
NINDS R01NS092651
Department of Veterans Affairs - Merit Review BX001148
PEDECIBA
SNI

Title: The pathogenic role of c-Kit+ mast cells in the spinal motor neuron-vascular niche in ALS

Authors: M. KOVACS[1], C. ALAMON[1], C. MACIEL[1], V. VARELA[1], S. IBARBURU[1], L. TARRAGO[1], P. H. KING[2,3], Y. SI[2,3], Y. KWON[2], O. HERMINE[4], L. BARBEITO[1], E. TRIAS[1]; [1]Inst. Pasteur, Montevideo, Montevideo, Uruguay; [2]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; [3]Birmingham Veterans Affairs Med. Ctr., Birmingham, AL; [4]Imagine Inst. INSERM UMR 1163, Hôpital Necker, Paris, France

Abstract: Degeneration of motor neurons, glial cell reactivity, and vascular alterations in the CNS are important neuropathological features of amyotrophic lateral sclerosis (ALS). Immune cells trafficking from the blood also infiltrate the affected CNS parenchyma and contribute to neuroinflammation. Mast cells (MCs) are hematopoietic-derived immune cells whose precursors differentiate upon migration into tissues. Upon activation, MCs undergo degranulation with the ability to increase vascular permeability, orchestrate neuroinflammation and modulate the neuroimmune response. However, the prevalence, pathological significance, and pharmacology of MCs in the CNS of ALS patients remain largely unknown. In autopsy ALS spinal cords, we identified for the first time that MCs express c-Kit together with chymase, tryptase, and Cox-2 and display granular or degranulating morphology, as compared with scarce MCs in control cords. In ALS, MCs were mainly found in the niche between spinal motor neuron somas and nearby microvascular elements, and they displayed remarkable pathological abnormalities. Similarly, MCs accumulated in the motor neuron-vascular niche of ALS murine models, in the vicinity of astrocytes and motor neurons expressing the c-Kit ligand Stem Cell Factor (SCF), suggesting an SCF/c-Kit-dependent mechanism of MC differentiation from precursors. Mechanistically, we provide evidence that fully differentiated MCs in cell cultures can be generated from the murine ALS spinal cord tissue, further supporting the presence of c-Kit+ MC precursors. Moreover, intravenous administration of bone marrow-derived c-Kit+ MC precursors infiltrated the spinal cord in ALS mice but not in controls, consistent with aberrant trafficking through a defective microvasculature. Pharmacological inhibition of c-Kit with masitinib in ALS mice reduced the MC number and the influx of MC precursors from the periphery. Our results suggest a previously unknown pathogenic mechanism triggered by MCs in the ALS motor neuron-vascular niche that might be targeted pharmacologically.

Disclosures: M. Kovacs: None. **C. Alamon:** None. **C. Maciel:** None. **V. Varela:** None. **S. Ibarburu:** None. **L. Tarrago:** None. **P.H. King:** None. **Y. Si:** None. **Y. Kwon:** None. **O. Hermine:** None. **L. Barbeito:** None. **E. Trias:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NINDS 5T32NS041218
Center for Neural Injury and Recovery (CNIR)

Title: Emergence of senescent cells in a toxin-induced mouse model of focal demyelination

Authors: *P. S. GROSS[1], Z. MANAVI[2], J. K. HUANG[1,2];
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Abstract: Multiple Sclerosis (MS) is a demyelinating and degenerative disease that is the number one neurological disability among young adults. Myelin degeneration results in denuded axons that consequently conduct signals less efficiently and eventually degenerate. In early stages of the disease, oligodendrocytes, the myelinating cells of the central nervous system, and their progenitors (OPCs), can regenerate myelin, resulting in effective remyelination. However, at later stages of the disease and with increasing age, remyelination becomes inefficient, leading to degeneration of axons and worsening of symptoms. One of the key characteristics of aging is the accumulation of senescent cells, which are apoptosis-resistant cells locked in permanent cell cycle arrest that secrete an inflammatory milieu termed the senescence associated secretory phenotype (SASP). Here, we hypothesize that the age-associated accumulation of senescent cells limits the ability of OPCs to remyelinate. Using a murine reporter line with a fluorochrome indicator expressed under the control of the $p16^{INK4a}$ locus (p16-tdt), a common marker of senescence, we found increased markers of senescent cells present after toxin-induced demyelination and throughout the process of remyelination. As early as 5 days post lesion (dpl), we found p16-tdt positive cells, with continued expression throughout and post- remyelination. Additionally, aged mice showed increased markers of senescence in the lesion compared to their young counterparts, suggesting the accumulation of senescent cells might contribute to their reduced remyelination. These results suggest that therapeutic targeting of cellular senescence, i.e. by senolytics or senomorphics, might promote remyelination at later stages of MS.

Disclosures: P.S. Gross: None. **Z. Manavi:** None. **J.K. Huang:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: H2020 MSCA-RISE project No 778405

Title: Calcium signal analysis protocols in studying the neuronal and astroglial response to ALS IgGs

Authors: *P. R. ANDJUS[1], A. KORENIć[1], M. MILOŠEVIć[1], D. BIJELIć[1], M. RADOJIčIć[1], K. ŽIVANčEVIć[1], B. E. KERMAN[2], A. ÇAPAR[3], S. D. ANTIC[4], W. VAN DRONGELEN[5]; [1]Univ. of Belgrade Fac. of Biol., Belgrade, Serbia; [2]Istanbul Medipol Univ., Istanbul, Turkey; [3]Istanbul Tech. Univ., Istanbul, Turkey; [4]Neurosci, UConn Hlth., Farmington, CT; [5]Dept Pediatrics, Univ. Chicago, Chicago, IL

Abstract: We aim towards the design of an innovative automated multifunctional device for diagnostics of neurodegenerative diseases. The main goal is to develop cellular models and procedures with immunoglobulin G (IgG) from patient sera for diagnostic protocols related to neurodegenerative diseases (particularly amyotrophic lateral sclerosis - ALS). We will report on the preliminary signal analysis protocols for Ca^{2+} imaging data obtained with fluorescent probes in rodent astrocytes or in neurons in culture. Time- lapse recordings were made with a CCD camera. The analysis was performed by developing machine learning protocols on astrocytes or applying cross-correlation analysis on neurons (van Drongelen W. Signal Processing for Neuroscientists: Introduction to the Analysis of Physiological Systems. Amsterdam: Elsevier, 2007). Raw data from primary rat cortical astrocytes were processed with Unsupervised Machine Learning algorithms in order to train a classifier to reliably predict a class for any given test trace. Clustering with Decision Trees along with Block Bootstrapping for time series to model the traces, as well as k-Nearest Neighbours classification gave us satisfactory preliminary results. After ALS IgG application the cross-correlograms of the Ca^{2+} signaling in hippocampal pyramidal neurons in culture revealed a synchronization that was apparent in highly correlated activity (corr. coeff. >0.9) across cell pairs at physiological delays. Control IgGs (commercial or from a healthy subject) have not shown similar highly synchronous behavior. Thus, in addition to an innovative technical solution for the use of cell models in a lab-on-chip device we have started developing robust signal analysis protocols on cells *in vitro* as induced by immune humoral factors that may serve for better stratification of patients for personalized medicine.

Disclosures: P.R. Andjus: Other; H2020 MSCA-RISE grant No 778405. **A. Korenić:** None. **M. Milošević:** None. **D. Bijelić:** None. **M. Radojičić:** None. **K. Živančević:** None. **B.E. Kerman:** None. **A. Çapar:** None. **S.D. Antic:** None. **W. van Drongelen:** None.

Poster

P318. Neuroinflammation: Neurodegeneration II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P318.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Akoya Biosciences

Title: Spatial phenotypic characterization of microglia via CODEX Multiplexed Imaging reveals sub-population-level relationships with pathogenic features in Alzheimer's Disease brains

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Abstract: Recent high-throughput transcriptomics and proteomics analysis have shown a remarkable heterogeneity of microglia phenotypes across various disease conditions. Differently from neurons, microglia appearance can be characterized as a continuum between ramified and amoeboid morphologies. The transcriptional and proteomic profiles associated to each degree of morphology are potentially multiple and dependent on the healthy/pathological conditions in consideration with sex, anatomical area of the brain, and disease stage. While single-cell RNA sequencing (scRNA-seq) has been instrumental in revealing microglia sub-populations, it is lacking in spatial information and phenotypic characterization. The scarcity of fresh human brain tissue imposes a bottleneck for RNA-seq studies, while paraffin-embedded tissues are widely available in most brain banks. Using Codex superplex imaging technology, we characterize microglia sub-population localization in relationship to the distance from neurodegenerative disease pathogenic features such as senile plaques and neurofibrillary tangles (NFT) in post-mortem AD brain samples and matching controls. Codex spatial phenotyping enabled us to characterize microglia that surround amyloid plaques and NFT-filled neurons in different brain regions of AD, dementia affected individuals with no signs of amyloid plaque deposits, and Tau mutant driven FTD affected individuals, and compare them with healthy age and sex matched individuals. This study allowed us to characterize the changes of microglia phenotype in the immediate neighborhood of pathogenic features. Such observation will be instrumental to understanding the role of several Late Onset Alzheimer’s Diseases associated genes and potentially identify novel drug targets.

Disclosures: J. Singh: None. **A. Bogachuk:** None. **N. Nikulina:** None. **A. Chiot:** None. **R. Woltjer:** None. **A. Crotti:** None. **O. Braubach:** None. **B. Ajami:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alpha5 patent rights. F. Consulting Fees (e.g., advisory boards); Tranquis therapeutic-advisory boards.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA013137
NIH Grant HD043680
NIH Grant MH106392
NIH Grant NS100624

Title: Synaptic dysfunction as a neural mechanism for HIV-1 associated apathy

Authors: *K. A. MCLAURIN, M. N. CRANSTON, H. LI, C. F. MACTUTUS, S. B. HARROD, R. M. BOOZE;
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Abstract: Individuals living with human immunodeficiency virus type 1 (HIV-1) exhibit an increased prevalence of neuropsychiatric comorbities (e.g., apathy) relative to their seronegative counterparts. Operationally, apathy is defined as a prominent decrease in self-generated voluntary or purposeful goal-directed behaviors; a construct which can be objectively measured in preclinical biological systems. HIV-1 associated apathy was examined by quantifying goal-directed behaviors, indexed using voluntary wheel running, in ovariectomized female HIV-1 transgenic (Tg; *n*=21) and F344/N control (*n*=26) animals. Analyses of voluntary wheel running were conducted at the microstructural level (i.e., parceling running behavior into fine-grained blocks to delineate the number and length of activity bouts) to elucidate genotype-dependent alterations in goal-directed behaviors. HIV-1 Tg animals exhibited prominent alterations in goal-directed behaviors, evidenced by a profound decrease in the number of running bouts relative to control animals; alterations which persisted during both the diurnal and nocturnal cycle. With regards to running bout length, HIV-1 Tg animals displayed an increased running bout length, relative to controls, during the diurnal cycle. During the nocturnal cycle, however, an altered development of running bout length was observed in HIV-1 Tg animals. Following the completion of voluntary wheel running, medium spiny neurons (MSNs) of the nucleus accumbens (NAc), an integral component of the fronto-striatal circuit, were examined as a potential neural mechanism underlying HIV-1 associated apathy. HIV-1 Tg animals displayed prominent alterations in MSNs of the NAc, evidenced by a population shift towards increased dendritic branching complexity and a more immature dendritic spine phenotype. Additionally, examination of the location of dendritic spines on MSNs in the NAc revealed decreased synaptic connectivity in HIV-1 Tg animals. Collectively, HIV-1 Tg animals displayed apathetic behaviors for a natural reward; behaviors which may be due, at least in part, to synaptic dysfunction in MSNs. Funded by: DA013137, HD043680, MH106392, NS100624.

Disclosures: K.A. McLaurin: None. **M.N. Cranston:** None. **H. Li:** None. **C.F. Mactutus:** None. **S.B. Harrod:** None. **R.M. Booze:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HD043680
MH106392
DA013137
NS100624
DA046258

Title: Abnormal intraneuronal β-amyloid accumulation in the hippocampus and prefrontal cortex and neurocognitive deficits in aging HIV-1 Tg rat and human HIV+ brain

Authors: *H. LI[1], K. MCLAURIN[1], C. MACTUTUS[1], B. LIKENS[1], S. L. CHANG[2], W. HUANG[2], R. BOOZE[1];
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Abstract: HIV-associated neurocognitive disorders (HAND) occur with increasing prevalence in aging HIV-1+ patients. To investigate the aging process in patients with HIV-1, we conducted two complementary studies 1) to elucidate abnormal protein aggregates (β-amyloid) in the aging HIV-1 transgenic (Tg) rat hippocampus and prefrontal cortex (PFC), and 2) to validate our findings on HIV+ human brain tissue from the HIV-associated neurocognitive disorder (HAND) patients. First, a longitudinal experimental design was utilized to evaluate the progression of temporal processing, assessed using gap prepulse inhibition (gap-PPI), in HIV-1 Tg and F344/N control rats from postnatal day (PD) 240 to PD 540. HIV-1 Tg rats exhibited prominent alterations in the progression of temporal processing relative to control rats; a deficit that was more pronounced in female HIV-1 Tg rats relative to males. Second, immunohistochemistry staining (IHC) and Western blot analysis were used to detect the expression of β-amyloid and amyloid precursor protein (APP) in the hippocampus and PFC of aging (14 month old) HIV-1 Tg and F344/N control rats. The data indicated that an abnormal intraneuronal β-amyloid accumulation was present in both the hippocampus and PFC in HIV-1 Tg rats when compared to F344/N control rats. Furthermore, significantly higher expression of APP was detected in the CA3 region of the hippocampus in F344/N control rats, relative to the HIV-1 Tg rats. We also used the DiOlistic labelling of dendritic spines to identify the intracellular consequences of β-amyloid accumulation. Finally, 9 autopsy HIV-infected cases (including hippocampus and PFC) from aging HIV-associated neurocognitive disorder (HAND) patients were used to assess the β-amyloid expression. The results showed intraneuronal β-amyloid expression both in the hippocampus and PFC, with no clear amyloid plaque pathology in these brain regions. Additionally, abundant Iba1+ cells co-localized with HIV-1 mRNA fluorescence signals,

providing strong evidence that microglia are the cell types harboring HIV-1 virus in the aging HAND brain. Overall, the abnormal intraneuronal β-amyloid accumulation in HIV+ will be critical for understanding the age-related neurocognitive deficits and assessing therapeutic interventions.

Disclosures: H. Li: None. **K. McLaurin:** None. **C. Mactutus:** None. **B. Likens:** None. **S.L. Chang:** None. **W. Huang:** None. **R. Booze:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ACTG NWCS 428
UAB CCTS

Title: Assessment of autophagy markers in HIV-seropositive participants with respect to antiretroviral therapy and neurocognitive status

Authors: *M. MCGRAW[1], E. ANDRAKA[1], S. OPSTEEN[1], L. PRICHARD[2], B. TIWO[4], E. T. OVERTON[2], G. R. CUTTER[3], S. HEATH[2], J. SHACKA[1];
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Abstract: Due to the success of antiretroviral therapy (ART), people with HIV (PWH) now exhibit near-normal life expectancies. However, a large percentage of PWH still suffer from several comorbidities including HIV-associated neurocognitive disorders (HAND). Previous studies have determined that the autophagy-lysosomal pathway (ALP) is dysregulated in peripheral blood mononuclear cells (PBMC) from PWH. Function of the ALP can be determined by assessment of autophagic flux, by quantifying the accumulation of autophagosomes in the presence or absence of lysosome inhibition. However, prior studies have not evaluated autophagic flux in specific populations of PBMCs, nor have they evaluated the impact of HAND or ART. This study tests our hypotheses that autophagic flux is compromised in PWH and is further compromised as a function of HAND. PBMCs were obtained from the following cohorts of participants in the ACTG-5303 study: without cognitive decline (N=12) before and after 48 weeks of ART (N=12), and with mild-moderate cognitive decline before and after ART (N=12). PBMCs were also obtained from HIV- participants (N=7). CD8+ T-cells were purified from PBMCs and then incubated for 24h in the presence or absence of chloroquine (CQ; 30 μM), a weak base that inhibits lysosome function, and in turn causes autophagosomes to accumulate.

Western blot analysis of cell lysates was performed to assess levels of LC3-II, a selective marker of autophagosomes, and p62, an ALP substrate. Levels of p62 and LC3-II in CD8+ T cell lysates were significantly lower in all HIV+ participant groups compared to those observed in HIV- participants. In HIV+ participant groups, levels of p62 were significantly higher after ART than those in ART-naïve participants, regardless of neurocognitive status. When assessing autophagic flux, LC3-II and p62 levels were significantly higher in CQ-treated lysates from ART-naïve, HIV+ participants compared to untreated lysates. This effect of CQ was not observed following ART. Taken together these results suggest that ART alters the ALP regardless of cognitive status. While our analysis of autophagic flux in CD8+ T cell lysates was unable to predict cognitive status, the observed effects of HIV on ALP function, and the additional effect of ART supports further investigation of the ALP as a means of monitoring disease progression.

Disclosures: M. McGraw: None. **E. Andraka:** None. **S. Opsteen:** None. **L. Prichard:** None. **B. Tiwo:** None. **E.T. Overton:** None. **G.R. Cutter:** None. **S. Heath:** None. **J. Shacka:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5T32GM081740
NIH Grant MH106292
NIH Grant DA013137
NIH Grant NS100624

Title: Dysbiosis in HIV-1 Transgenic Rats: Potential Treatment of HAND through the Gut-Brain-Microbiota Axis

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Abstract: HIV-1 associated neurocognitive disorders (HAND) have been found to be associated with microbial translocation and dysbiosis. These changes are associated with the progression and severity of HAND symptoms in humans. HIV-1 transgenic (Tg) rats are used to study the neurocognitive deficits associated with HAND but the role the gut-brain-microbiota axis contributes remains unknown. S-equol is a metabolite produced by the gut when ingesting specific phytoestrogens and has been found to be neurorestorative. This study was a 2x2 factorial design where we used 16S rRNA gene sequencing and profiling to determine if there was a difference in microbiome composition between adult HIV-1 Tg rats (n = 21) and F344/N control

rats (n = 21), before and after S-equol treatment. HIV-1 Tg rats contain 7 of the 9 HIV-1 viral proteins (env, tat, rev, vif, vpr, vpu, nef) and are noninfectious due to not having gag and pol proteins. Treatment consisted of S-equol (four 0.5mg pellets) or four sucrose control pellets per animal per day for 70 days. Stool samples were collected before and after treatment, with intestinal samples being collected only after. Alpha diversity analysis between HIV-1 Tg vs. control rats at baseline was approaching significance ($p<0.051$) with beta diversity analysis indicating a clear difference in phylogenetic composition but not abundance ($p<0.001$). At the genus level, baseline differences were found between HIV-1 Tg rats vs. controls with Alloprevotella and Bacteroides being increased in the HIV-1 Tg rats. Weighted UniFrac analysis of phylogenetic differences and taxonomic abundance provided evidence of a significant effect of S-equol in HIV-1 Tg rats ($p<0.007$) and controls ($p<0.047$). After treatment, Alloprevotella was decreased in HIV-1 Tg animals, with a relative abundance closer to controls. The intestinal tissue samples did not display a difference in beta diversity analysis or at the phylum level, but the genus level indicated a larger relative abundance of Fusobacterium in the HIV-1 Tg rats vs. controls. The decrease in Alloprevotella supports the use of S-equol as a treatment for HAND due to Alloprevotella being gram-negative and possessing Lipopolysaccharide (LPS), an inflammatory endotoxin found on the outer membrane of gram-negative bacteria. LPS is an important measure of microbial translocation due to its ability to activate microglia, increasing inflammation and contributing to worsening cognitive deficits associated with HAND. Improving the microbiome and reducing the circulating LPS through S-equol treatment would provide a mechanism by which to treat HAND through the gut-brain-microbiota axis.

Disclosures: M. Rodriguez: None. **K.A. McLaurin:** None. **C.F. Mactutus:** None. **R.M. Booze:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH087332
NIH MH104131
NIH MH105330
NIH DA052209
NIH DA026306 (P5)

Title: Elucidating the role of ephrin-b1 in interferon beta mediated neuroprotection of HIV associated neurocognitive disorder (HAND)

Authors: *J. KOURY[1], H. SINGH[1,2], S. SUTLEY[1], D. FOK[1], B. GELMAN[3], I. ETHELL[1], M. KAUL[1,2];
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Abstract: 37 million people worldwide are diagnosed with Human Immunodeficiency Virus (HIV). Of these 37 million, an estimated 15-55% of people living with HIV develop HIV-associated neurocognitive disorder (HAND). Previous work in ours and other labs identifies a transient increase of interferon beta (IFNβ), an anti-inflammatory and antiviral type 1 interferon, preceding any behavioral or neuropathological signs in the HIVgp120 transgenic mouse and SIV models, suggesting a neuroprotective role for IFNβ in early HIV infection. We have previously shown that exogenous intranasal exposure of HIVgp120tg animals with IFNβ is sufficient to confer neuroprotection. Here, we describe the ability of IFNβ to regulate an important cell surface transmembrane ligand, ephrin-b1, in the CNS. Neuronal ephrin-b1 has been well studied and implicated in neuronal development, recruitment of NMDA receptors and synaptic plasticity. However, the regulation of ephrin-b1 as well as ephrin-b1's role in HIV has not been well characterized. In this study, we identify a significant positive correlation between type 1 interferon master regulator, IRF7, and Ephrin-b1 mRNA in the cortex of HIV+ Patients. In addition, we describe significant inverse correlations between Ephrin-b1 mRNA in the cortex of HIV+ Patients with both neurocognitive domain scores and neuronal gene expression. In a transgenic NeuroHIV mouse *in vivo* model, HIVgp120tg, we show in cortex and hippocampus that abrogating IFNβ signaling decreases RNA expression of ephrin-b1. Conversely, intranasal treatment with IFNβ induces ephrin-b1 protein expression in a cell type specific manner within the hippocampus and partially in the cortex in non-transgenic control animals while paradoxically, IFNβ treatment of HIVgp120tg mice leads to a robust decrease in ephrin-b1. Taken together, this data suggests that IFNβ may be an important regulator of ephrin expression, and that the direction of this regulation is contingent on the presence of a viral component, such as HIVgp120.

Disclosures: J. Koury: None. **H. Singh:** None. **S. Sutley:** None. **D. Fok:** None. **B. Gelman:** None. **I. Ethell:** None. **M. Kaul:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1R41MH116921-01
2R44MH119621-02

Title: High-content analysis and Kinetic Image Cytometry identify toxic and epigenotoxic effects of HIV antiretrovirals on human iPSC-neurons and primary neural precursor cells

Authors: A. S. SMITH[1], S. ANKAM[1], C. FARHY[2], L. FIENGO[2], R. C. B. BASA[1], *K. GORDON[1], A. V. TERSKIKH[2], K. L. JORDAN-SCIUTTO[3], **J. H. PRICE**[1], P. M. MCDONOUGH[1];
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Abstract: Despite viral suppression due to combination antiretroviral therapy (cART), HIV-associated neurocognitive disorders (HAND) continue to affect half of people with HIV, suggesting that certain antiretrovirals (ARVs) may contribute to HAND. We examined the effects of nucleoside/nucleotide reverse transcriptase inhibitors tenofovir disproxil fumarate (TDF) and emtricitabine (FTC) and the integrase inhibitors dolutegravir (DTG) and elvitegravir (EVG)) on viability, structure, and function of glutamatergic neurons derived from human induced pluripotent stem cells (hiPSC-neurons), and primary human neural precursor cells (hNPCs), which are responsible for neurogenesis. Using the Microscopic Imaging of Epigenetic Landscapes (MIEL) assay, we found that TDF decreased hNPC viability and changed the distribution of histone modifications that regulate chromatin packing, suggesting that TDF may reduce neuroprogenitor pools important for CNS development and maintenance of cognition in adults. Using automated digital microscopy and image analysis (high content analysis, HCA), we found that DTG, EVG, and TDF decreased hiPSC-neuron viability, neurites, and synapses after seven days of treatment. Analysis of hiPSC-neuron calcium activity using kinetic image cytometry demonstrated that DTG and EVG also decreased the frequency and magnitude of intracellular calcium transients. Longer ARV exposures and simultaneous exposure to multiple ARVs increased the magnitude of these neurotoxic effects. We then tested if co-culturing the hiPSC-neurons with hiPSC microglia prior to treatment with ARVs could yield a protective effect on neuronal health. Inclusion of microglia in the cultures attenuated the toxic effect of DTG and EVG on neuronal calcium activity. We are working to develop co-culture techniques with hiPSC-neurons, astrocytes, and microglia to advance the model for ARV toxicity to resemble the CNS more closely. This study establishes human preclinical assays that can screen potential ARVs for CNS toxicity and develop safer cART regimens and HAND therapeutics.

Disclosures: A.S. Smith: None. **S. Ankam:** None. **C. Farhy:** None. **L. Fiengo:** None. **R.C.B. Basa:** None. **K. Gordon:** None. **A.V. Terskikh:** None. **K.L. Jordan-Sciutto:** None. **J.H. Price:** None. **P.M. McDonough:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA32444
DA15014

Title: Role of Divalent Metal Transporter 1 in Morphine-Mediated Upregulation of Neuronal Ferritin Heavy Chain

Authors: *E. IROLLO[1,2], R. BRANDIMARTI[1,2], O. MEUCCI[1,2,3];
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Abstract: HIV-associated neurocognitive disorders (HAND) persist in about 50% of people with HIV on antiretroviral therapies, highlighting an important clinical and scientific challenge. HAND symptoms are also worsened by opioids and other abused substances, which may be at least partially due to the ability of these substances to alter synaptic function in brain regions involved in learning and memory and executive function. Indeed, our previous work demonstrated that opioids reduce dendritic spine density in cortical neurons through a unique pathway. We first found that morphine inhibits the dendritic spine promoting action of the CXCL12/CXCR4 chemokine signaling axis by upregulating the iron storage protein ferritin heavy chain (FHC). Further analysis showed that morphine released iron stored in neuronal endolysosomes to the cytoplasm, and this was required to upregulate FHC. Here, we tested if this mechanism involves the divalent metal transporter 1 (DMT1), a well-known iron transporter expressed on endolysosomes. First, we validated antibodies for the different DMT1 isoforms (+/-IRE) in HEK293 cells expressing recombinant DMT1+/-IRE proteins or silenced for DMT1. We then optimized conditions to detect DMT1 isoforms (+/-IRE) in rat cortical neurons and found that the majority of DMT1 was expressed in the cytoplasm. Immunofluorescent staining showed colocalization of DMT1 isoforms and the endolysosomal marker lysosomal-associated membrane protein 1 (LAMP1), suggesting that both DMT1 isoforms are expressed in endolysosomal vesicles. To assess the potential contribution of DMT1 in morphine-mediated upregulation of FHC, we pre-treated neuronal cultures with ebselen, a potent inhibitor of the iron transport of DMT1. Ebselen prevented FHC upregulation in morphine treated cultures, suggesting that DMT1 does play a role in morphine-mediated endolysosomal iron efflux. To further validate the contribution of DMT1, we are currently testing the effect of a specific genetic inhibition through viral-mediated silencing of DMT1 in cortical neurons. Overall, these studies suggest that DMT1 is involved in morphine-mediated regulation of CNS iron metabolism and that therapeutic interventions targeting the brain iron metabolism might be beneficial for individuals affected by neurocognitive disorders.

Disclosures: E. Irollo: None. **R. Brandimarti:** None. **O. Meucci:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Glycogen synthase kinase-3 inhibition rescues sex-dependent contextual fear memory deficit in HIV-1 transgenic mice

Authors: *S. MOIDUNNY[1], M. A. BENNEYWORTH[4], D. J. TITUS[4], E. BEUREL[2], U. KOLLI[1], J. MEINTS[4], R. JALODIA[1], S. RAMAKRISHNAN[1], C. M. ATKINS[3], S. ROY[1]; [1]Dept. of Surgery, [2]Dept. of Psychiatry and Behavioral Sciences; Dept. of Biochem. and Mol. Biol., [3]The Miami Project to Cure Paralysis, Dept. of Neurolog. Surgery, Univ. of Miami Miller Sch. of Med., Miami, FL; [4]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Background and Purpose. A significant number of HIV-1 patients on antiretroviral therapy develop HIV-associated neurocognitive disorders (HAND). Recent evidence indicate that biological sex may regulate HAND pathogenesis, but the mechanisms remain unknown. We investigated synaptic mechanisms associated with sex differences in HAND, using the HIV-1-transgenic 26 (Tg26) mouse model. **Experimental approach.** Contextual- and cue-dependent memories of male and female Tg26 mice and littermate wild-type mice were assessed in a fear conditioning paradigm. Hippocampal electrophysiology, immunohistochemistry, western blot, qRT-PCR and ELISA techniques were used to investigate cellular, synaptic and molecular impairments. **Key results.** Cue-dependent memory was unaltered in both male and female Tg26 mice when compared to wild-type mice. Interestingly, male, but not female, Tg26 mice showed deficits in contextual fear memory. Consistently, only male Tg26 mice showed depressed hippocampal basal synaptic transmission and impaired LTP induction in area CA1. These deficits in male Tg26 mice were independent of hippocampal neuronal loss and microglial activation but were associated with increased HIV-LTR mRNA expression, reduced hippocampal synapsin-1 protein, reduced BDNF mRNA and protein, reduced AMPA glutamate receptor (GluA)1 phosphorylation levels and increased glycogen synthase kinase-3 (GSK3) activity. Importantly, selective GSK3 inhibition using 4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione increased levels of synapsin-1, BDNF and phosphorylated-GluA1 proteins, restored hippocampal basal synaptic transmission and LTP, and improved contextual fear memory in male Tg26 mice. **Conclusion and Implications.** Sex-dependent impairments in contextual fear memory and synaptic plasticity in Tg26 mice are associated with increased GSK3 activity. This implicates GSK3 inhibition as a potential therapeutic strategy to improve cognition in HIV-1 patients.

Disclosures: S. Moidunny: None. **M.A. Benneyworth:** None. **D.J. Titus:** None. **E. Beurel:** None. **U. Kolli:** None. **J. Meints:** None. **R. Jalodia:** None. **S. Ramakrishnan:** None. **C.M. Atkins:** None. **S. Roy:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 MH087332
NIH R01 MH104131
NIH R01 MH105330
NIH R01 DA052209

Title: Blocking LOX: a Doorway to Neurotoxic Damage in HIV-1 gp120 Associated Brain Injury

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Abstract: Individuals infected with human immunodeficiency virus (HIV)-1 often develop HIV-associated neurocognitive disorders (HAND). Studies in our laboratory indicate that p38 MAPK is essential for the neurotoxic phenotype of HIV gp120-stimulated macrophages and microglia, as well as the induction of neuronal apoptosis triggered by macrophage-derived toxins and proinflammatory molecules. Microarray analyses of human macrophages stimulated with HIV envelope protein gp120 and of brains of transgenic mice expressing the viral gp120 under the control of a modified GFAP promotor in astrocytes (gp120tg mice) suggested the involvement of cysteinyl leukotrienes (cysLTs) in HIV neurotoxicity.
Leukotrienes (LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4) are the product of the 5-lipoxygenase (5-LOX) metabolism of arachidonic acid. CysLTs (LTC_4, LTD_4, and LTE_4) are formed by the addition of cysteine derivatives. Two distinct CysLT receptors exist, CysLTR1 and CysLTR2 (Kanaoka & Austen reported a third CysLTR). CysLTs are most notably researched for their role in the pathophysiology of asthma but their role in the brain is largely unknown.
We have found that cysteinyl leukotrienes (CysLTs) are released by both HIV-1 infected and gp120-stimulated macrophages, and knockdown of p38 MAPK down-regulates cysteinyl leukotriene synthase (LTC4S). Herein, we describe the characterization of both CysLTR1 deficiency and LTC4S deficiency in gp120tg mice by immunofluorescence staining, RNA analysis, analyte detection, and behavioral studies, which suggests a critical role for the CysLT pathway in NeuroHIV.

Disclosures: N. Yuan: None. **A.B. Sanchez:** None. **C. DeRosieres:** None. **K. Medders:** None. **M. Kaul:** None.

Poster

P319. Mechanisms of HIV Associated Neurocognitive Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P319.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R21NS120806
NIH NCATS TL1TR003019
NIH NIGMS T32GM8339

Title: Neuro-immune interactions after HIV-1 infection in human microglia-containing cerebral organoids

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Abstract: While combined antiretroviral therapy (cART) has reduced the incidence and severity of HIV-related AIDS, up to half of HIV-positive patients must still grapple with neurological complications. These complications can eventually escalate into HIV-Associated Neurocognitive Disorder (HAND). There is an urgent need to develop a more representative human model system to study the molecular and cellular mechanisms underlying HIV-related neuropathology. We have developed human microglia-containing cerebral organoids (hmCOs) derived from human pluripotent stem cells (hPSC) to investigate how HIV-1 infection leads to microglial dysregulation, persistent inflammation, and neuronal functional changes. We derived primitive macrophage precursor cells (PMPs) from hPSCs that underwent further microglia differentiation before infecting with replication-competent macrophage-tropic HIV-1. We detected productive infection by detecting p24 in a time-dependent manner as well as changes in inflammatory cytokine/chemokine secretion. RNAseq analysis revealed enrichment of interferon type I signaling pathways. Finally, we incorporated HIV-infected microglial cells into cerebral forebrain organoids. We found that microglia successfully integrate and interact with neurons within hmCOs and assessed viability and mobilization of HIV-1 infected microglial cells. Further analyses will reveal the impact of HIV-1 infection on microglial and neuronal physiology. We anticipate that this platform will help facilitate new insights into pathogenesis and novel therapeutics for neuroAIDS.

Disclosures: A.J. Boreland: None. **H. Lin:** None. **A. Stillitano:** None. **R. Xu:** None. **R.P. Hart:** None. **P. Jiang:** None. **Z.P. Pang:** None. **A.B. Rabson:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research
Canada Foundation for Innovation
Alberta Innovates

Title: Transcutaneous spinal cord stimulation alters excitability between multiple converging segments of the spinal cord

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Abstract: Transcutaneous spinal cord stimulation (tSCS) has the potential to facilitate improved sensorimotor rehabilitation by non-invasively modulating circuitry of the spinal cord. Currently, little is known about how cervical or lumbar tSCS influences the excitability of spinal and corticospinal networks and whether synergistic effects of multi-segmental tSCS occur between remote segments of the spinal cord. Two studies aimed to determine the extent to which spinal (H-reflex) and corticospinal circuitry (motor evoked potential [MEP]) can be modulated by cervical, lumbar, or combined tSCS. Study participants were seated in a recumbent cycling system. During study 1 (n=13), lower limb H- reflexes were elicited in the soleus muscle, while in study 2 (n=14), upper limb H-reflex and MEP amplitudes were evaluated in the flexor carpi radialis muscle. These were assessed during 2 tasks: 1) Limbs held in a static position; 2) During rhythmic cycling of the arms (study 1) or legs (study 2). In both studies, conditions included 1) No tSCS, 2) tSCS applied to the cervical enlargement, and 3) tSCS applied to the lumbar enlargement. In study 2, tSCS was also simultaneously applied to the cervical and lumbar enlargements. Interestingly, tSCS of the cervical cord with arms static significantly suppressed lumbar cord excitability as indicated by a 22.9% reduction in soleus H-reflexes amplitude, while lumbar tSCS facilitated H-reflex amplitude in the upper limbs by 11.1% and combined cervical and lumbar tSCS increasing the facilitation to 19.6%. A convergence of combined tSCS significantly increased MEP amplitude by 19.7% compared to No tSCS. As hypothesized, soleus H-reflex amplitude was significantly suppressed by 19.2 % during arm cycling relative to arms static (without tSCS) while leg cycling alone suppressed the FCR H-reflex by 13.6%. When combined with either arm or leg cycling, tSCS was unable to alter excitability across both studies indicating that in neurologically intact individuals where interlimb coordination and corticospinal tract are intact, the effect of arm or leg cycling on cervico-lumbar coupling and corticospinal drive was not impacted significantly with the tSCS intensity used. These studies demonstrate that tonic activation of spinal cord networks alters excitability over multiple segments of the spinal

cord while multiple sites of tSCS converge to enhance modulation of spinal reflex and corticospinal pathways. This highlights the potential for engaging cervical and lumbar spinal cord networks through tSCS to enhance the effectiveness of rehabilitation interventions.

Disclosures: T.S. Barss: None. **B. Parhizi:** None. **V.K. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR
University Hospital Foundation
Canada Foundation for Innovation

Title: Laser-microfabricated, smart polymer intraspinal multielectrodes reduce foreign body responses in mixed glial cell cultures and generate functional responses in the pig spinal cord

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Abstract: Intraspinal microstimulation (ISMS) is an emerging neural prosthetic approach for restoring standing and walking after spinal cord injury (SCI) by applying electrical stimulation within the spinal cord. Historically, ISMS has been implemented with hair-like microwire electrodes with one active site each (Holinski et al. 2016), or very stiff silicon multielectrodes (MEs) (Pikov et al. 2020) that exacerbate foreign body responses (FBR). This project focused on developing flexible, smart polymer MEs to improve the targeting efficiency and biocompatibility of ISMS implants. Three-channel flexible polymer MEs were fabricated from a polydimethylsiloxane (PDMS) composite substrate, modified with 1.75% w/w dimethylsiloxane-(ethylene oxide) block copolymer (PDMS-PEG BCP) (Gökaltun et al., 2019), and platinum-iridium (Pt/Ir: 80%/20%) foil using a nanosecond laser. Electrochemical parameters were assessed in phosphate-buffered saline (PBS) using electrochemical impedance spectroscopy (EIS) and voltage transients (VT). Glial cell adhesion and reactivity were assessed using immunocytochemistry in mixed mouse glial cell cultures at 7 and 14 days for MEs prepared with unaltered or BCP-modified PDMS substrates. Implant buckling forces were assessed through standard compression tests. In vivo assessments of ME electrochemical properties were performed in 4 domestic pigs and compared to those of 50 µm Pt/Ir microwires commonly used for ISMS. The capacity of the new MEs in producing functional leg movements was also tested.

ME stimulating sites exhibited significantly greater electrochemical impedance values than microwire electrodes in PBS, displayed higher charge injection limits than microwire electrodes in vivo, and were capable of evoking sufficient joint torques and kinematics for producing functional movements. The buckling force of the MEs was significantly lower than the microwire electrodes, demonstrating improved flexibility. MEs with PDMS-PEG surface modifications also showed a marked reduction in glial cell adhesion in vitro compared to unaltered PDMS, suggesting that reduced FBRs may also be seen in chronic in vivo implants. In conclusion, laser-fabricated, smart polymer MEs may be viable alternatives to current ISMS implants in the future.

Disclosures: D.A. Roszko: None. **C. Tsui:** None. **S. Mirkiani:** None. **N. Tyreman:** None. **D. Wilson:** None. **K.G. Todd:** None. **V.K. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research
Canada Foundation for Innovation
Faculty of Medicine and Dentistry, University of Alberta

Title: Robot-assisted investigation of the neural mechanisms underlying arm function

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Abstract: Introduction: Daily activities necessitate engagement of the arms and are not limited to simple isolated reaching movements. Spinal cord injury (SCI) often affects arm function, and complex movements including those that require bimanual manipulation concurrent with cognitive and sensory processing may be more clinically relevant for improving rehabilitation outcomes than current strategies. Nonetheless, the choice of a rehabilitation intervention needs to be based on an understanding of the underlying organizations in the neural correlates of arm movement. The goal of this project was to identify the influence of more complex movements on motor processes associated with the planning and execution of arm movements.**Methods:** The underlying mechanisms of motor control of goal-directed reaching movements were investigated in neurologically intact (NI) study participants. Cortical activity was recorded via electroencephalographic electrodes while the participants performed multiple movements: 1)

unimanual visually-guided reaching (VGR) where they were instructed to move their virtual hand to peripheral targets surrounding a central location (using KINARM exoskeleton); 2) reverse VGR whereby they moved their hand to peripheral targets by moving their arm in the reversed direction to add a cognitive load; 3) uncooperative bimanual VGR in which each arm performed similar center-out reaching movements to peripheral targets simultaneously; 4) cooperative bimanual reaching movements where the participants moved a ball placed on a horizontal bar to peripheral targets through cooperative movement of the two arms, each holding one end of the bar; and 5) reverse VGR versions of the uncooperative and cooperative bimanual movements. Beta band (13-30Hz) cortical activity associated with sensorimotor processes was computed using spectral power for C1, C3, FC1, and FC3 electrodes.**Results:** Uncooperative bimanual reaching movements increase the magnitude of activity during both planning and execution stages in motor and pre-motor areas compared to both unimanual and cooperative bimanual movements. Interestingly, moving in the reverse direction, both in unimanual and bimanual movements, reduces the level of activity in the motor and pre-motor cortex during both planning and execution stages.**Significance:** This project demonstrates, for the first time, the alterations in motor control of goal-directed arm movements with increasing motor and cognitive demands. Cortico-muscular coherence and cortical functional connectivity will be investigated to explore the neural mechanisms associated with different spectral power level across complex tasks.

Disclosures: B. Parhizi: None. **A. Dineros:** None. **G. Sivadasan:** None. **V. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: US Department of Defense Spinal Cord Injury Program
Canadian Institutes of Health Research and Canada Foundation for Innovation
CLO and DAR were supported by an NSERC Graduate Scholarship and a Walter Johns Scholarship
PF was supported by a University of Alberta Recruitment Scholarship
AT was supported by a Canada Vanier Graduate Scholarship and an Alberta Innovates Graduate Studentship
VKM is a Canada Research Chair in Functional Restoration

Title: Safety of Mapping the Motor Networks in the Spinal Cord

Authors: *C. O'SULLIVAN[1], S. MIRKIANI[1], D. A. ROSZKO[1], P. FARIDI[1], D. S. HU[2], D. G. EVERAERT[2], A. TOOSSI[1], R. KANG[3], D. FANG[3], N. TYREMAN[2], A. N. DALRYMPLE[1], K.

ROBINSON[7], R. R. E. UWIERA[4], H. SHAH[8], R. FOX[5], P. E. KONRAD[9], V. K. MUSHAHWAR[6];
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Abstract: Introduction: Epidural spinal cord stimulation was proposed for restoring walking after severe spinal cord injury, and 4 individuals were able to step overground for distances up to 91m using their arms to weight-bear. With better targeting of the locomotor networks residing in the ventral horn of the lumbar enlargement, walking may be improved. These networks were successfully mapped in pre-clinical models; however, their location is unknown in humans. This study developed a procedure for mapping these locomotor networks and evaluated its safety in adult Yucatan pigs. **Methods:** Pigs underwent pre-surgical assessments including walking kinematics and reflex function in the legs. Under anesthesia, clinically-approved microelectrodes (125µm) were inserted into the ventral horn using a stereotactic frame and ultrasound guidance. To facilitate electrode penetration through the pia mater, small cuts were made using an arachnoid knife. The pigs were divided into 2 groups, an experimental and a sham control group. The experimental group received up to 12 electrodes insertions in one side of the spinal cord and the resulting movements induced by microstimulation were documented. The sham control received pial cuts only. Four weeks post-surgery, the kinematics and reflexes were reassessed in all animals and compared to pre-surgical measures. The animals were then euthanized, and their spinal cords extracted for immunohistochemical analysis. **Results:** Post-surgically, the experimental and sham control groups had similar balance and gait deficits over the first 2 weeks. Four weeks post-surgery, reflexes returned to pre-surgical levels and walking kinematics were similar to pre-surgical measures in all but one animal in the experimental group. Immunohistochemical analyses revealed only very few electrode tracks (<10% of all penetrations). Disruption to the dorsal surface of the spinal cord caused by the pial cuts was much more visible and at times extended 1mm into the spinal cord. **Conclusion:** Following an adequate recovery period, the reflexes and kinematics return to baseline despite multiple cord penetrations during the intraoperative procedure. Damage caused by electrode insertions is negligible; however, damage caused by the pial insertions is notable. A less disruptive technique should be developed or smaller diameter microelectrodes with sharper tips that allow for penetration through the pia mater that have safely been used in intraspinal microstimulation, should be utilised. The results suggest that intraoperative insertion of electrodes into the spinal cord is potentially safe for future application in humans to map the locomotor networks.

Disclosures: C. O'Sullivan: None. **S. Mirkiani:** None. **D.A. Roszko:** None. **P. Faridi:** None. **D.S. Hu:** None. **D.G. Everaert:** None. **A. Toossi:** None. **R. Kang:** None. **D. Fang:** None. **N. Tyreman:** None. **A.N. Dalrymple:** None. **K. Robinson:** None. **R.R.E. Uwiera:** None. **H. Shah:** None. **R. Fox:** None. **P.E. Konrad:** None. **V.K. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canada Foundation for Innovation (CFI)
Canadian Institutes of Health Research (CIHR)
PF is supported by University of Alberta FGSR scholarships (Alberta Graduate Excellence Scholarship 2020 & The University of Alberta Master's Entrance Scholarship 2020)

Title: Intraspinal microstimulation to restore functional walking after complete chronic spinal cord injury

Authors: *P. FARIDI[1], V. K. MUSHAHWAR[2];
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Abstract: The overall goal of this project is to develop a technique to restore over-ground walking in animals with complete spinal cord injury (SCI) using intraspinal microstimulation (ISMS). ISMS entails implanting micro-electrodes into the lumbosacral enlargement of the spinal cord to produce synergistic activations of the legs. To date, ISMS has produced in-place stepping in cats with complete chronic SCI (Saigal et al, 2004), and also long distances of over ground walking in anesthetized cats (Holinski et al, 2016). However, two unanswered questions need to be addressed prior to translating ISMS to humans. 1) Can over-ground walking be achieved with ISMS in cats with chronic SCI in which varying degrees of spasticity and exaggerated reflex responses, commonly seen after SCI in humans, are present? 2) Can the stimulation patterns be automatically predicted for each individual since each injury is different, and each person walks differently from others?To restore functional walking, a biologically-inspired state control strategy was designed and successfully bench-tested for coordinating the activation of neural networks and resulting muscle synergies. The strategy included time-based transitions within different phases of the step cycle, resembling the rhythmic alternations of legs, which then can be modified through pre-defined rules, using feedback from force plates built in a walkway and angular velocities from gyroscopes placed on the hindlimbs of the animal. To answer the second question, reinforcement learning in combination with Pavlovian control, inspired by classical conditioning, are implemented to learn the feedback signals' patterns and produce predictive a walking strategy. This predictive method was implemented previously, for the first time, in cats with incomplete SCI, where feedback signals of the intact limb were used to predict the state of walking for the paralyzed limb, moved through ISMS (Dalrymple et al, 2020).To test the feasibility of the predictive controller for a complete SCI model, a musculoskeletal model of cat hindlimbs was developed in MATLAB which used Newtonian

dynamics and Hill-type muscle properties (Hill et al, 1938). Walking was divided into 4 sub-phases and muscle synergies in each sub-phase were activated with a constant level of motoneuronal excitation, leading to a muscle activation pattern that mimics the activation generated by ISMS. This project is a critical step towards producing personalized bipedal walking. If successful in both simulation and in-vivo testing, the system may change the lives of many people living with SCI, giving them the capability to walk independently.

Disclosures: P. Faridi: None. **V.K. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: U.S. Department of Defense

Title: Strain-relief system for mechanically stable intraspinal microstimulation

Authors: *S. MIRKIANI[1,2], D. A. ROSZKO[1,2], D. WILSON[3,2], N. TYREMAN[3,2], P. TROYK[5,2], R. FOX[6,2,1], V. K. MUSHAHWAR[4,2,1];
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Abstract: Intraspinal microstimulation (ISMS) is a novel technique that directly stimulates locomotor circuits in the ventral horn of the spinal cord using penetrating microelectrodes. While ISMS has demonstrated promising results in restoring standing and walking after spinal cord injury in rats and cats, design modifications are required for large animal models, and eventually humans, due to their higher range of motion. In this study, we demonstrate a strain-relief system for ISMS that can minimize the stresses on the electrodes. Microwires (25 µm Pt-Ir, 80%-20%, insulated with polyimide) forming the leads to each microelectrode were coiled around a tungsten rod (D= 100 µm) using a custom-built coiling device. The coiled microwires (length of coiled region= 4 mm, OD= 160 µm) were then dip-coated with polydimethylsiloxane (PDMS) and cured using a heat gun (100 °C for 10 minutes). The uncoiled ends of 16 leads were then bundled, inserted in a Silastic tube (OD: 500 µm, ID: 30 µm), and coiled around a tungsten rod (D= 200 µm). The tube was then filled with PDMS, unwound, and placed in the oven at 100 °C for 1 hour to cure the PDMS. A PDMS tab (250 µm thick) was incorporated in the Silastic tube using a spin-coater before insertion of the microwires. The tab was used to anchor the ISMS device on the spinal cord. Uniaxial tension and electrochemical impedance spectroscopy (EIS)

tests were performed on the coiled cable containing the 16 microwires to analyze their mechanical and electrical properties. The cable was stretched up to 40% of strain and the effect of elongation on its impedance was evaluated. The system was also tested in vivo in five freshly euthanized domestic pigs to assess the feasibility of anchoring the ISMS system to the spinal cord, dura mater, and spinous process of the L3 vertebra. The elongation force (at 40% of strain) was 701± 12,8 mN (mean ± SD, 6 repeated measurements). The capacitance of the coiled cable at 100 Hz and 1 kHz was calculated to be 0.033±0.0014 nF, and 0.029±0.0002 nF, respectively (mean ± SD, 6 repeated measurements). Applying up to 40% strain on the coiled leads had no significant effect on the impedance, capacitance, and resistance of the wires ($p>0.05$). Pig cadaver experiments confirmed the appropriateness of the size of the coiled microwires and coiled cable in the limited subarachnoid space of the spinal cord. Moreover, the ISMS array was successfully secured to the spinal cord. The increase in extension capability of the ISMS array obtained by incorporating the coiled cable offers more reliability and safety of the electrodes by minimizing the tethering forces on the spinal cord.

Disclosures: S. Mirkiani: None. **D.A. Roszko:** None. **D. Wilson:** None. **N. Tyreman:** None. **P. Troyk:** None. **R. Fox:** None. **V.K. Mushahwar:** None.

Poster

P371. Spinal Cord Injury: Training, Rehabilitation, and Repair III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P371.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canada Foundation for Innovation
Canada Research Chairs
Canadian Institutes for Health Research
Craig Neilsen Foundation
National Primate Research Center at U. Washington

Title: Comparative anatomy and functional neurophysiology of the lumbar spinal cord and locomotor networks: implications for spinal cord neuromodulation

Authors: A. TOOSSI[1], D. EVERAERT[2], B. PARHIZI[1], S. MIRKIANI[1], C. O'SULLIVAN[1], S. I. PERLMUTTER[3], ***V. MUSHAHWAR**[2];
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Abstract: Electrical spinal cord neuromodulation is an emerging approach for restoring locomotor function after severe spinal cord injury; however, the methods for most effectively

activating the locomotor networks which reside in the lumbar enlargement remain elusive. The goal of this project was to identify the location and functional organization of the locomotor networks across multiple species to provide a systematic understanding of their activation through neuromodulation. Several studies were performed: 1) Immunohistochemistry was used to identify the distribution of cells activated by two spinal cord neuromodulation approaches: epidural spinal cord stimulation (eSCS) and intraspinal microstimulation (ISMS). 2) Electrophysiology was used to map the spinal cord in cats, pigs and non-human primates. 3) MRI was used to develop atlases of the lumbar cord in rats, cats, pigs, non-human primates and humans. Preliminary studies in anesthetized pigs used the activity marker cfos to compare the distribution of neurons activated in the lumbar cord by eSCS and ISMS. Bilateral eSCS and ISMS electrodes were implanted and electrical stimuli were delivered to one side, eliciting small but perceptible movements in the ipsilateral limb. With eSCS, the distribution of activated neurons was concentrated in laminae I-IV with sparse scattering in the rest of the gray matter, while with ISMS, the distribution was concentrated in laminae VI, VII and medial IX. The spinal cords in cats, pigs and non-human primates were mapped to investigate the functional organization of locomotor networks in the ventral horn. Single electrodes were advanced in a 3D grid throughout the lumbar enlargement in cats and non-human primates, and arrays of microwire electrodes were implanted in pigs. For each site, stimulation threshold and the evoked limb kinematics, force and EMG activity in the ipsilateral limb were obtained and used to construct 3D functional maps. The maps were similar between species, with minor differences in the size allocation of various components of the map in non-human primates. The findings suggest that eSCS activates locomotor networks indirectly, likely through reflex pathways, while ISMS activates locomotor-related networks more directly. They also demonstrate that the functional organization of locomotor networks is preserved across mammalian species and is likely to be similar in humans. To facilitate more accurate modeling of the locomotor networks, detailed 3D atlases of the location of the locomotor networks were constructed based on high-resolution MRIs of spinal cords extracted post-mortem from 5 species commonly used in neuromodulation experiments.

Disclosures: A. Toossi: None. **D. Everaert:** None. **B. Parhizi:** None. **S. Mirkiani:** None. **C. O'Sullivan:** None. **S.I. Perlmutter:** None. **V. Mushahwar:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.01

Topic: D.03. Somatosensation – Pain

Support: NIH/NIDCR DE029187-01S2

Title: Identification of trigeminal sensory neuronal types innervating masseter muscle

Authors: K. A. LINDQUIST;
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Abstract: Understanding masseter muscle (MM) innervation is critical for themanagement of head and neck disorders and performing masseteric nerve transplantation/transfer. This study identifies and characterizes trigeminal (TG) sensory neuronal groups innervating MM (MM TG neurons), including muscle fibers, masseteric fascia, and tendons. We identified 9 distinct types of MM TG neurons using a combination of patch clamp electrophysiology and immunohistochemistry on TG neurons back-traced from reporter mouse MM. Of these neurons, 24% belonged to nonpeptidergic IB-4+/TRPA1- or IB-+/TRPA1+ groups, while two TRPV1+ small-sized neuronal groups were classified as peptidergic/CGRP+. One small-sized CGRP+ neuronal group had a unique electrophysiological profile and were recorded from Nav1.8- or trkC+ neurons. The remaining CGRP+ neurons were medium-sized, could be divided into Nav1.8-/trkC- and Nav1.8low/trkC+ clusters, and showed large 5HT-induced current. The final two MM TG neuronal groups were trkC+ and had no Nav1.8 and CGRP. MM TG neurons dis not have TRPV1+/CGRP-(somatostatin+), tyrosine hydroxylase+ (C-LTMR), TRPM8+, MrgprA3+ or trkB+ (Aδ-LTMR) groups. We next examine distribution of different afferent fibers in MM. Use of Nav1.8-YFP reporter, and labeling with CGRP, TRPV1, neurofilament heavy chain (NFH) and pgp9.5 antibodies showed that muscle fibers and tendons were exclusively innervated by medium-to-large neurons. These nerves were myelinated, either CGRP+/NFH+ or CGRP-/NFH+ and mainly distributed in tendon and at junctions of deep-middle-superficial parts of MM. Overall, the data presented here demonstrates that MM is innervated by a distinct subset of TG neurons, which have unique characteristics and innervation patterns compared to DRG neurons and fibers.

Disclosures: K.A. Lindquist: None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.02

Topic: D.03. Somatosensation – Pain

Support: NIDCR Grant DE026749
NIDCR grant NS064022
NEI Core grant EY08098

Title: Multi-unit recording in the parabrachial in rats after whisker pad injection of varicella zoster virus

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Abstract: Multi-unit recording in the parabrachial in rats after whisker pad injection of varicella zoster virus**Rebecca S. Hornung, Ph.D.**[1], Paul R. Kinchington, Ph.D.[2], Phillip R. Kramer, Ph.D.[11]Texas A&M University College of Dentistry, Dallas, TX, United States[2]University of Pittsburgh, Department of Ophthalmology, Pittsburgh, Pennsylvania, United States
Background: The parabrachial nucleus (PBN) modulates the affective-motivational component of orofacial pain. The central amygdala (CeA) has inhibitory GABAergic neurons that project to and inhibit the lateral PBN. In a rat model of neuropathic pain, the neuronal activity of the lateral PBN is amplified. Stimulating the CeA-PBN pathway suppresses acute pain, while inhibiting the pathway induces nociceptive behaviors. Our lab utilizes a chronic post-herpetic neuralgia animal model where human varicella zoster virus (VZV) is injected into the whisker pad of the rat. Injection of VZV to the whisker pad results in herpes zoster-associated (HZ; shingles) pain that can last for at least 3 months. In humans, the most common complication of HZ is postherpetic neuralgia (PHN) and PHN is observed more following HZ of the orofacial region. In rodents, VZV-induced pain results in hypersensitivity like PHN in humans. Our long-term goal is to test the hypothesis that inhibiting GABAergic neurons within the CeA that project to the lateral PBN will result in increased neuronal activity and increased affective-motivational behavior in rats. Since the PBN is located deep within the brain, we first had to investigate whether implantation of a microelectrode to the PBN could detect neuronal activity. **Methods**: GAD67 transgenic adult male Long Evans rats were infused with an adeno-associated virus (AAV) containing a Cre-dependent G inhibitory designer receptor exclusively activated by designer drugs (DREADD) to the amygdala. Following infusion, a microelectrode was implanted to the depth of the PBN. Four weeks later, VZV was injected into the left whisker pad. One week later, affective-motivational behavior was tested using a place escape avoidance paradigm (PEAP) and neuronal activity was recorded with a Tucker Davis RZ10x.**Results**: Results indicate neuronal activity of neurons within the PBN of adult male rats in-vivo can be measured. **Conclusion**: Results suggest that modulation of PBN neuronal activity events of adult rats can be measured in-vivo.**Acknowledgements**: This study was funded by NIDCR grant (DE026749) to PRKramer, NIDCR grant (NS064022) to PRKinchington, and NEI core grant (EY08098).

Disclosures: R. Hornung: None. **P. Kinchington:** None. **P. Kramer:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.03

Topic: D.03. Somatosensation – Pain

Support: NIH NIDCR F31DE030677

Title: Trigeminal inputs directly relay nociceptive information to rostral ventromedial medulla pain-modulating neurons

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Abstract: The brain regulates nociceptive processing through descending projections from the brainstem to the spinal and trigeminal dorsal horns. These endogenous pain-modulating circuits can amplify or suppress pain-related signals. Under normal conditions, these circuits maintain a balance between facilitation and inhibition of pain, but become dysregulated in chronic pain states. The output of this pain-modulating system, via the rostral ventromedial medulla (RVM), has been extensively studied. Bidirectional pain control from this region is mediated by two, physiologically defined cell classes, "ON-cells" and "OFF-cells," that respectively facilitate and inhibit nociceptive transmission. However, sensory inputs to RVM remain to be fully defined. Indirect inputs from the dorsal horn via the parabrachial complex were recently shown to convey nociceptive information to RVM and contribute to the sensitization of RVM neurons in persistent inflammatory pain. However, there also is anatomical evidence for a direct input from the dorsal horn to RVM. The purpose of this study was to define direct and indirect inputs to RVM from the trigeminal dorsal horn, which relays sensory input from the face and head. Using a combination of single-cell recording in RVM with optogenetic activation of trigeminal inputs expressing channelrhodopsin, light-evoked responses of identified ON- and OFF-cells were recorded in lightly anesthetized male and female Sprague-Dawley rats. Stimulation of the direct trigeminal-to-RVM projection resulted in a significant increase in ON-cell firing rate and a significant decrease of OFF-cell firing rate. NEUTRAL-cells, RVM neurons without a known role in pain modulation, showed no change in activity following activation of this pathway. Conversely, archaerhodopsin-induced inhibition of trigeminal terminals in RVM attenuated the noxious-evoked activity of ON- and OFF-cells. A similar approach revealed an indirect relay of trigeminal information to RVM through the parabrachial complex. These data demonstrate a direct trigeminal relay that conveys nociceptive information to RVM, with a parallel indirect pathway through the parabrachial complex.

Disclosures: C.C. De Preter: None. **M.M. Heinricher:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.04

Topic: D.03. Somatosensation – Pain

Title: Orofacial hyperalgesia (is) associated with selective deletion of trigeminal lemniscal patterning in Prrxl1 mutant mouse

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Abstract: Trigeminal neuropathic pain, a frequently debilitating orofacial pain more prevalent with age and in women, is difficult to manage, resistant to conventional treatment and contributes significantly to a poor quality of life. Prrxl1, a paired homeodomain transcription factor, which is indispensable for the development of patterning in the trigeminal lemniscal pathway is also expressed by primary sensory neurons in dorsal root ganglia and their central targets in the dorsal horn. Prrxl1 deletion disrupts patterning of primary sensory afferent fibers into the spinal dorsal horn and abolishes patterning in the whisker-to-barrel cortex pathway. It has long been known that Prrxl1 deletion is associated with hypoalgesia to body stimulation; we now report that it is also associated with hyperalgesia to stimulation of the orofacial region as well as displaying significantly extended bouts of orofacial grooming. Taken together, these observations suggest a hitherto unreported contribution of Prrxl1 to the organization of orofacial nociception. The rodent trigeminal system has been implicated in a variety of active sensing behaviors, including object recognition, texture discrimination and ingestive behavior. Although absence of patterning is associated with disruption of these behaviors, its involvement in nociception has not been previously reported and the impact of nociception on these behaviors is poorly understood. To elucidate these effects, facial mechanical hyperalgesia was assessed using von Frey monofilaments applied to the mystacial (whisker) pad. Similarly, spinally mediated mechanical as well as thermal nociception was evaluated at the hind paw and tail respectively and compared to trigeminally mediated responses obtained from the face in Prrxl1 KO, Barrelless (BRL, an animal lacking cortical patterning) and wild-type (WT) CD-1 mice. It was found that, Prrxl1 KO mice exhibited hypoalgesia of the hind paws while displaying hyperalgesia of the orofacial region which was significantly attenuated by subcutaneous administration of the anti-inflammatory analgesic Carprofen (5.0 mg/kg). The frequency of orofacial grooming was also determined through 24-hour continuous video monitoring. Prrxl1 mice displayed significantly more grooming bouts and groomed for a longer duration compared to BRL and WT. These results suggest (1) that the disruption of lemniscal patterning in Prrxl1 KO mice is selectively associated with orofacial nociception (2) that these altered behaviors are reflective of a chronic pain condition and (3) that this mutant may be a useful, non-invasive model of orofacial pain.

Disclosures: G. Cataldo: None. **P. Feinstein:** None. **H.P. Zeigler:** None. **J.C. Brumberg:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.05

Topic: D.03. Somatosensation – Pain

Support: R01NS099245
R01NS069568

Title: Responses of trigeminal ganglion neurons to noxious and innocuous stimuli remain unchanged with aging

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Abstract: With age come changes in sensory function. The incidence of chronic pain increases with age (PMID: 30212442). In humans, pain thresholds increase with age, while pain tolerance is reported to either decrease (PMID: 15876494) or remain unchanged (PMID: 28159611). Of particular concern is that pain in the elderly, particularly among those with dementia, is underreported (PMID 26955380). Because neural activity can provide insight into sensory processing and functional outcomes, one approach to developing pain metrics and understanding mechanisms of pain in aging is to compare responses along the somatosensory pathway, starting with the trigeminal ganglion. We recorded responses from trigeminal ganglion neurons of adult Fischer 344 (F344) rats aged 4-6 months (7 males and 10 females), and aged F344 rats ages 19-25 months (4 male and 10 female rats). We used single-cell, extracellular, urethane-anesthetized recordings to assess trigeminal ganglion neuron responses to both noxious mechanical stimulation and to innocuous, high-frequency whisker deflection. There was no age difference in the magnitude of responses to noxious mechanical stimulation (n= 22 adult neurons, n=24 aged, Mann-Whitney U=255, p=0.85). The fidelity of responses to high-frequency, “pink noise” vibrissa stimulations are highly reproducible in rats (PMID: 15218153). There was no effect of age on the reproducibility of these responses, as quantified by the correlation coefficient between trials (n=19 control male cells, n=13 aged male cells, n=12 control female cells, n=13 aged female cells; $F(1,53)=0.26$, $p=0.61$). However, there was a main effect of sex, with males having higher correlation coefficients between stimulation trials than females ($F(1,53)=17.6$, $p=0.0001$, eta squared=0.25, large effect size), suggesting that responses in males are more reproducible. These data suggest that changes in age-related sensory functions are unlikely related to changes in primary afferents, and might reflect changes in downstream CNS structures.

Disclosures: O. Uddin: None. **A. Keller:** None. **M. Plank:** None. **R. Masri:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.06

Topic: D.03. Somatosensation – Pain

Support: R01 DE26139
T32 DE14318
F30 DE31177

Title: 5-ht preferentially releases complement peptides c3a from peripheral tissues of women compared to men, leading to the sexual dimorphic increase in trpv1 activities in trigeminal sensory neurons

Authors: *M. TRAM, M. ESKANDER, Q. LI, P. CHANG, N. B. RUPAREL, K. M. HARGREAVES;
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Abstract: Numerous studies have documented sex and gender differences in pain, but the underlying mechanisms remain unclear. Our group has demonstrated that serotonin (5-HT) sensitizes human dental pulp to capsaicin (CAP)-dependent CGRP release in biopsies from women, but not men (Loyd et al Pain 153:3061). Here, we determine the mechanisms mediating this effect. Methods: Participants provided human third molar dental pulps after standard extraction protocol under IRB approval. Dental pulp superfusion was conducted as previously described (Loyd et al.). Soluble factors were subjected to 2D-DIGE proteomics. Activity of cultured trigeminal (TG) neurons was measured by whole cell patch recording or calcium imaging (Fura2 AM). Data were analyzed by ANOVA. Results: The application of 5-HT to female, but not male, human dental pulp released a soluble factor that sensitized TG neurons to I_{CAP} or CAP-induced [Ca]i. This soluble factor was a protein and not a lipid as the CAP sensitizing effect was destroyed by incubation with proteinase K, but not lipid removal using Clean Ascites. 2D-DIGE identified complement peptide C3a (C3a) as being differentially released from female compared to male superfused dental pulp. The CAP-sensitizing effect of the conditioned media from superfused dental pulp was abolished by incubation with anti-C3a antibody or application of a C3a receptor angatonist (SB290157). The application of C3a peptide to TG neurons triggered significant increases in TG neurons to I_{CAP} or CAP-induced [Ca]i. Finally, the CAP-sensitizing effects of C3a on TG neurons was abolished by pre-treatment with either a PKC inhibitor (bisindolymaleimide) or a SRC inhibitor (A419259). Conclusions: 5-HT preferentially releases C3a from peripheral tissues of women compared to men, leading to the sexual dimorphic increase in TRPV1 activities in trigeminal sensory neurons.

Disclosures: M. Tram: None. **M. Eskander:** None. **Q. Li:** None. **P. Chang:** None. **N.B. Ruparel:** None. **K.M. Hargreaves:** None.

Poster

P383. Trigeminal Circuits and Processing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P383.07

Topic: D.03. Somatosensation – Pain

Support: NIH NICDR DE025970 to DLA
Diversity Supplement to DJC
Texas Woman's University Center for Student Research
Texas Woman's University Alumni Association
Texas Woman's University Quality Enhancement Program

Title: Sex Differences in Stress-Exacerbated Orofacial Pain and Glial Density in Ascending Trigeminal Pain Pathways

Authors: *D. CANTU, A. LOPEZ-RAMIREZ, N. SANTOS, E. RODRIGUEZ, D. AVERITT; Biol., Texas Woman's Univ., Denton, TX

Abstract: Psychological stress influences craniofacial and orofacial pain disorders, many of which disproportionately affect women. Unfortunately, most preclinical studies investigating the effects of stress on orofacial pain are reported in male rodents. It is unclear whether stress exacerbated orofacial pain occurs in females. Probable mechanisms involved in stress exacerbated orofacial pain may implicate corticosterone levels and astrocyte activity. Studies indicate that stress decreases Glial Fibrillary Acid Protein in the brain (GFAP). Other studies have shown that astrocytes increase one day after exposure to Complete Freund's Adjuvant (CFA; inflammatory agent) into the masseter muscle. Moreover, more cortisol is present in women experiencing pain when compared to men. Overall, our study aims to determine whether exposure to sub-chronic stress (the forced swim test; FST) can alter mechanical sensitivity at the inflamed vibrissal pad in male and female rats, correlate exposure to the FST with corticosterone levels, and visualize astrocytes in the brainstem of these rats. We hypothesized that FST evokes greater orofacial pain behaviors in females which correlates to increased blood corticosterone levels and increased astrocyte density in ascending trigeminal pain circuitry. Mechanical sensitivity was recorded in adult Sprague-Dawley male and female rats using the von-Frey method. Rats received one injection of CFA into the right vibrissal pad (RVP). Twenty-four hours later, orofacial allodynia was confirmed. All rats were subjected to either three consecutive days of the FST or sham test. Mechanical allodynia was re-assessed on 1, 4, and 8, and 11 days following the FST or sham paradigms. On day 8, all rats received the trans-synaptic tracer wheat-germ-agglutinin into the RVP. On day 11, mechanical allodynia was re-assessed immediately, followed by perfusion fixation. Baseline blood corticosterone was measured via ELISA and re-assessed following FST. The trigeminal ganglia and brainstem were extracted, sectioned, and analyzed by confocal microscopy. We report that injecting CFA into the RVP evoked significant mechanical allodynia in males and females. Females display greater allodynia one, four, and eight days after FST when compared to males. Our data also indicate that inflamed males exposed to the FST express more GFAP in the brainstem when compared to sham.

Conversely, female brainstem expressed comparable GFAP after FST or sham conditions. Altogether, our data indicate sex differences in the effects of stress on mechanical allodynia in the orofacial region, which may be one contributing mechanism to the prevalence of orofacial pain in women.

Disclosures: D. Cantu: None. **A. Lopez-Ramirez:** None. **N. Santos:** None. **E. Rodriguez:** None. **D. Averitt:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.01

Topic: D.07. Vision

Support: NIH NS 109978
NIH NS 107968

Title: Spatial modulation of dark versus bright stimulus responses in the mouse visual system

Authors: *B. WILLIAMS[1], J. DEL ROSARIO[2], T. MUZZU[3], K. PEELMAN[2], S. COLETTA[2], E. K. BICHLER[2], A. SPEED[2], L. MEYER-BAESE[2], A. B. SALEEM[3], B. HAIDER[2]; [1]Biomed. Engin., [2]Georgia Tech. and Emory Univ., Atlanta, GA; [3]Univ. Col. London, London, United Kingdom

Abstract: A fundamental task of the visual system is to respond to both increases and decreases of luminance (ON and OFF responses). OFF responses are stronger, faster, and more salient than ON responses in primary visual cortex (V1) of cats and primates and primates (Yeh et al., 2009; Jin et al., 2011; Xing et al., 2010), but in ferrets and mice ON responses can be stronger, weaker, or balanced in comparison to OFF responses (Smith et al., 2015; Smith and Hausser, 2010; Polack and Contreras, 2012; Jimenez et al., 2018). These discrepancies could arise from differences in species, experimental techniques, or stimulus properties, particularly retinotopic location in the visual field, as has been speculated (Smith et al., 2015); however, the role of retinotopy for ON/OFF dominance has not been systematically tested across multiple scales of neural activity within species during awake vision. Here, we measured OFF versus ON responses across 150° of visual space (spanning both monocular and binocular visual fields) with silicon probe and whole-cell patch-clamp recordings in awake mouse V1 and lateral geniculate nucleus (LGN). We analyzed local field potential (LFP), extracellular spiking, and subthreshold membrane potential responses to brief, spatially localized visual stimuli (flashed bars or squares). For all recordings, we quantified the relative ratio of ON versus OFF responses, facilitating comparisons across recording modalities.We found that OFF responses dominated in the central visual field, whereas ON and OFF responses were more balanced in the periphery. These

findings were broadly consistent across local field potential (LFP), spikes, and subthreshold membrane potential in V1, and were aligned with spatial biases in ON/OFF responses in LGN. Our findings reveal that retinotopy may provide a common organizing principle for spatial modulation of OFF versus ON processing in mammalian visual systems.

Disclosures: B. Williams: None. **J. Del Rosario:** None. **T. Muzzu:** None. **K. Peelman:** None. **S. Coletta:** None. **E.K. Bichler:** None. **A. Speed:** None. **L. Meyer-Baese:** None. **A.B. Saleem:** None. **B. Haider:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.02

Topic: D.07. Vision

Support: NIH Grant EY018875

Title: Figure-ground representation of horizontal and vertical disparity mechanisms in human visual cortex

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Abstract: Previous work has shown that signals evoked by horizontal and vertical disparities are stronger in the presence of a disparity reference (Norcia et al, 2017). Therefore, both mechanisms could support perceptual figure-ground segmentation that depends on edge discontinuities between two disparity-defined regions. However, only horizontal disparities give rise to vivid percepts of depth. This suggests that reference effects are likely to arise via different cortical mechanisms for the two disparity types. Here, we used fMRI to probe the cortical distribution of responses to horizontal and vertical disparities, particularly in relation to a zero-disparity surround. Participants (N=11) viewed circular dynamic random-dot stereograms that consisted of a central 4-degree disk embedded within a 12-degree annulus. The central region could be segmented from its surround on the basis of one of four disparity types. These were crossed or uncrossed horizontal disparity, and corresponding vertical disparity conditions. Disparity amplitude of the central disk was modulated between +/- 12 and 0 arcmin, at a rate of 2 Hz. In a fifth condition, the central disk was modulated between 0 disparity and binocularly uncorrelated dots. Stimuli were presented in blocks of 12 seconds and were interspersed with a zero-disparity baseline condition consisting of a uniform field of random dots. During scanning, participants monitored centrally placed nonius lines and pressed a button when they noticed a colour change. fMRI scans were pre-processed and aligned to high-resolution, segmented anatomical scans using fmriprep. Response amplitudes were estimated using a standard GLM

approach in voxels restricted to the grey matter. Preliminary results indicate that horizontal disparity conditions (crossed and uncrossed) evoked robust responses in a network of areas that spanned early visual and lateral occipital cortex. Responses to vertical disparity were weaker and clustered in early visual areas, highlighting differences in the cortical representation of the two cues. In some participants, we also measured negative BOLD responses at the edges of the retinotopically-defined central disk representation. This was more pronounced for horizontal disparity, suggesting the presence of a suppressive mechanism that may support image segmentation via higher level form-from-disparity cues.

Disclosures: M. Kaestner: None. **A.M. Norcia:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.03

Topic: D.07. Vision

Support: NSF CRCNS Grant 1822598
NIH Grant EY031059

Title: Contrast versus luminance in retina and visual cortex

Authors: *L. DYBALLA[1], M. HOSEINI[3], M. RUDZITE[5], G. D. FIELD[6], S. W. ZUCKER[2], *M. P. STRYKER[4];
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Abstract: The traditional view is that visual cortex is concerned with contrast and discounts changes in background luminance as compared with retina. We studied responses of neurons in retina and primary visual cortex (V1) of mouse to stimuli including moving gratings and optic flows built from either dark or light dots or short line segments (Dyballa et al *PNAS* 2018). Stimuli subtending ~60° x 40° were presented for 1.25 sec separated by 0.75 sec mid-gray screens. We asked whether average luminance matters when comparing retinal with cortical responses.
Two interleaved stimulus sets were identical except for the background. In one, the flow stimuli background was the mid-gray of the inter-stimulus interval (equal background, *eqbg*), leading to a change of 9-10% in the space-average luminance. In the other, the space-average luminance of the entire stimulus field was adjusted to a constant (equal luminance, *eqlum*) within 0.5%; i.e., the background was slightly lightened when the dots in the flow were dark, and darkened when the dots were bright.
Most cortical cells appeared to respond similarly to the two stimulus sets, as if stimulus structure

mattered but not the background change, while the responses of most retinal ganglion cells appeared to differ between the two conditions. Machine learning algorithms confirmed this quantitatively. A manifold embedding of neurons to the two stimulus sets was constructed using diffusion maps. In this manifold, the responses of the same cell to *eqlum* and *eqbg* stimuli were significantly closer to one another for V1 rather than for the retina. Geometrically, the median ratio of the distance between the responses of each cell to the two stimulus sets as compared to the distance to the closest cell on the manifold was 3.5 for V1 compared to 12.7 for retina. Topologically, the fraction of cells for which the responses of the same cell to the two stimulus sets were connected in the diffusion map datagraph was 53% for V1 but only 9% for retina; when retina and cortex were co-embedded in the manifold, these fractions were 44% and 6%. While retina and cortex differ on average, it will be intriguing to determine whether particular classes of retinal cells behave more like V1 neurons, and vice versa.

Disclosures: L. Dyballa: None. **M. Hoseini:** None. **M. Rudzite:** None. **G.D. Field:** None. **S.W. Zucker:** None. **M.P. Stryker:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.04

Topic: D.07. Vision

Support: NSF Grant NSF-1854158

Title: Standardizing methods for analyzing the P100 latency of visually evoked potentials

Authors: *F. M. INGRAM[1], J. C. WAGNER[2], S. A. BEARDSLEY[2,3];
[1]Dept. of Biol. Sci., [2]Dept. of Biomed. Engin., Marquette Univ., Milwaukee, WI; [3]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Visually evoked potentials (VEPs) represent changes in the brain's electrical activity due to a visual stimulus. VEPs are often measured using electroencephalography (EEG) and can be characterized by the latency of the P100 peak as an indicator for the onset of visual processing in the occipital lobe. VEP P100 latency is widely used for diagnosing damage along the optic tract, such as with optic neuritis, and can provide insight into damage elsewhere in the brain, as with Multiple Sclerosis (MS) patients. However, lack of consensus in the literature has resulted in a failure to create a criterion for classifying latencies. The goal of this study is to establish a standardized method for measuring the P100 latency. In a pilot study, 5 young adults with normal or corrected-to-normal vision (2 males, ages 19-25 years) participated in a 2 Hz pattern reversal checkerboard experiment while 64-channel EEG was recorded. Two different check sizes (1.05° and 1.43°) were examined for monocular and binocular viewing conditions across

200 reversals. After typical EEG preprocessing, channel level analysis of the P100 latency was quantified using two different methods: peak latency and percent area. Peak latency was defined as the time the signal reached maximum amplitude whereas the percent area method used the timepoint where the signal reached 50% of the area under the curve within the P100 onset/offset window. Latencies for both methods were calculated for all conditions using the signals from the left (O1), medial (Oz), and right (O2) EEG occipital channels averaged across reversals. A three-way ANOVA was used to identify differences between method, check size, and eye condition. No significant differences in the P100 latency were found between conditions ($p > 0.05$), but observable trends in the variability of measured latencies were present for check size and analysis method. Increased variability occurred with the larger check size (e.g., right eye variance peak method, large: 98.0 ms^2 vs. small: 14.7 ms^2) and when using the peak latency method of analysis (vs. right eye variance percent area method, large: 11.8 ms^2). Reducing variability in latency calculations is important, especially in populations whose EEG signals may be more susceptible to noise. This percent area method reduces the variability of latency measurements by averaging the effects of noise across the P100 interval. Future work will evaluate the changes in P100 latency with age and for patient populations, such as those with MS. Standardizing VEP latency analysis around a single sensitive and robust method will facilitate comparisons across studies and EEG systems.

Disclosures: F.M. Ingram: None. **J.C. Wagner:** None. **S.A. Beardsley:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.05

Topic: D.07. Vision

Support: NSF IIS-1350990
NEI/NIH EY025403

Title: Binocular integration as nonlinear mixing: how binocular neurons in primary visual cortex preserve eye-specific information for downstream visual processing

Authors: *E. S. CHENG, F. BARTSCH, D. A. BUTTS;
Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: The visual cortex integrates the visual information from the two eyes into a single “fused” percept. The straightforward way to combine the information from each eye is to use the relative shift between the two images, known as the binocular disparity, to bring the images into register. Indeed, a fraction of neurons in primary visual cortex (V1) are tuned to specific disparities, and it is commonly thought that such disparity signals are necessary to appropriately

integrate eye-specific information in V1 or downstream areas. Paradoxically, however, a large fraction of neurons in V1 are binocular but have little-to-no sensitivity to disparity. Here, we explore an alternative mode of binocular integration: based on the hypothesis that "binocular untuned" neurons support the preservation of eye-specific information in the form of nonlinear mixtures. We support this hypothesis by analyzing accurate data-driven models that are based on macaque V1 neurons recorded in a previous study. In these models, disparity tuning in V1 arises from linear summations of left- and right-eye information; and conversely, the responses of binocular untuned neurons represent nonlinear mixtures of left and right eye information. We performed population-level simulations of each V1 neuron in a binocular rivalry paradigm with independent images presented to each eye. Using population decoding of these simulated responses, we find that rather than obscuring visual information from each eye, such nonlinear mixtures preserve eye-specific information. Conversely, disparity-tuned neurons - which linearly mix information from the two eyes - were less able to preserve eye-specific information. However, the performance of these disparity-tuned neurons in the decoding paradigm could be "rescued" by modifying the models to replace linear with nonlinear summation of left- and right-eye information, leaving all other aspects of the models the same. Our results demonstrate that a large fraction of V1 neurons preserve eye-specific visual information, even though they are binocular. Furthermore, we suggest that the formatting of eye-specific information in the form of nonlinear mixtures is a feature, not a bug, to support downstream processing that requires eye-specific information: for example, in tasks involving the reconstruction of three-dimensional geometries from the two-dimensional images available to our two eyes.

Disclosures: E.S. Cheng: None. **F. Bartsch:** None. **D.A. Butts:** None.

Poster

P479. Processing of Contrast, Form, and Color II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P479.06

Topic: D.07. Vision

Support: SH was funded by a joint Wellcome Trust/ NIH studentship (ref 100931/Z/13/Z; http://www.wellcome.ac.uk)
NEI/NIH Grant EY025403
Intramural research program at NEI/NIH
NSF IIS-1350990

Title: Amplification of disparity selectivity by spatial convolutions in the primary visual cortex

Authors: *F. BARTSCH[1], S. HENRIKSEN[2,3], J. C. READ[3], B. G. CUMMING[2], D. A. BUTTS[1];
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Abstract: Our two eyes see slightly shifted versions of the visual world, which are combined within the brain into a single fused percept. Bringing the two images into register requires knowing the binocular disparity (shift between the images in each eye), and some neurons in primary visual cortex (V1) are exquisitely sensitive to the disparity of binocularly presented stimuli. However, while existing models of binocular integration can explain V1 disparity tuning in the context of simple stimuli such as drifting gratings, it is not clear whether these mechanisms generalize to more natural stimuli. Here, we present a model for V1 processing that accounts for its disparity tuning in the context of complex spatiotemporal visual stimuli. It is based on the canonical mechanism of linear summation of spatiotemporally filtered inputs from each eye - common to most previous models of disparity tuning - but adds a novel mechanism for disparity amplification via spatial convolutions. We use a data-driven modeling framework applied to recordings from V1 neurons in awake macaques, where random bar stimuli aligned to each neuron's receptive field were presented using a haploscope with randomly changing disparity. Unlike previous models of V1 disparity tuning, our model is able to almost completely reproduce the disparity tuning of a wide variety of binocular V1 neurons to complex stimulus patterns: explaining nearly all of the disparity-driven response of V1 neurons that were significantly disparity tuned (mean disparity-R^2 = 88%, n=11, with many neurons almost perfectly explained). Furthermore, because the model concurrently captures each neuron's spatiotemporal feature selectivity, such a model also generalizes to non-disparity-tuned V1 neurons (mean explainable R^2 = 56%, n=50). Furthermore, based on these models fit to data, we derive an understanding of how disparity processing of specialized disparity-tuned neurons is amplified by spatial convolution. Specifically, the spatial convolution allows identical processing to be performed across space, resulting in sensitivity to the correlations present in natural visual input: with disparity typically correlated over large spatial scales (due to the depth structure of the visual scene) and spatiotemporal patterns changing on much finer spatial scales. Convolutional processing thus amplifies the correlated disparity signals across space while averaging out the signals driven by spatiotemporal patterns. In addition to offering an elegant explanation for disparity processing, such a convolutional strategy - observed broadly in V1 neurons - is likely at play in amplifying selectivity to a variety of other visual features.

Disclosures: F. Bartsch: None. **S. Henriksen:** None. **J.C. Read:** None. **B.G. Cumming:** None. **D.A. Butts:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.01

Topic: H.02. Perception and Imagery

Support: NIH/NIMH R21MH107052-01A1
NSF BCS-1658560
NSF BCS-1658078

Title: Functional parcellation of the STS: A comparison of four atlases and six social perception localizers

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Abstract: BACKGROUND: In the human neuroimaging field, there is significant effort to develop brain parcellation algorithms that automatically draw borders between distinct functional regions. The benefits to automated brain parcellation include dimensional reduction and consistent functional mapping across individual participants without conversion to volumetric templates such as MNI. Ideally, parcellations that strongly align with functionally localized regions would eliminate the need for collecting additional localizer scans, reducing burdens on the human participant and standardizing approaches across fMRI studies. The superior temporal sulcus (STS) covers a large expanse of cortex stretching from the anterior temporal lobe to posterior parietal cortex, and is host to numerous cognitive functions in social and cognitive domains (Liebenthal et al., 2014). Currently available atlas schemes, optimized for whole-brain parcellation, yield very different boundary definitions within the STS (Patel et al., 2020). We therefore compared these parcellations on their ability to differentiate core social cognitive tasks that yield complex activation patterns on the human STS. METHOD: We evaluated four publicly available automated parcellation schemes on their efficacy in discriminating the following tasks: biological motion perception, perception of agency from Heider-Simmel type animations, theory of mind as induced by false belief tasks, stationary and dynamic face perception, and audiovisual speech perception. Efficacy of atlas parcellation was evaluated using leave-one-subject-out classification on task labels, with the classifier trained on metrics of homogeneity of activation and functional connectivity within each parcel. RESULTS: All tasks were readily classified for all four atlas parcellations across a variety of homogeneity metrics, evidence that each of the six tasks yield unique multivariate activation patterns apparent even at the parcel level. In particular, the homogeneity of effect sizes within the parcels had robust information that yielded improved classification, even superior to homogeneity of mean effect sizes and within-parcel connectivity. CONCLUSIONS: These findings demonstrate that qualitatively different activation maps from pSTS localizers yield multivariate patterns across atlas parcels that are readily differentiated quantitatively. Further work is required to identify what features promote optimal task-identification and thus have potential to improve the utility of atlases in replacing functional localizers.

Disclosures: B.C. Hackney: None. **D.A. Stehr:** None. **J.A. Pyles:** None. **E.D. Grossman:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.02

Topic: H.02. Perception and Imagery

Support: NSF BCS-1658560
NSF BCS-1658078

Title: Functional connectivity of the pSTS in the action observation network (AON) modulated by attention to action features during action and goal inference, an fMRI experiment

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Abstract: Background. Neural mechanisms of action understanding are associated with the action observation network (AON), which includes the posterior superior temporal sulcus (pSTS), the inferior frontal gyrus (IFG), and bilateral intraparietal lobule (IPL). Whereas the pSTS is linked specifically to perceptual representations of action kinematics, the IPL and IFG are proposed to represent the implied goals and outcomes of the observed action. Theoretical models propose, and empirical findings support the notion, that representations in the IFG modulate and restructure perceptual representations in the pSTS via feedback routed through the IPL. The IFG and IPL, however, are large and functionally heterogeneous brain regions with complex organization. Moreover, there exist multiple pathways by which the IFG may interact with sensory cortical systems during action recognition. The goal of this study is to evaluate the fine-grained organization of parcels functionally connected to the pSTS during action recognition and that are modulated by the attentive state during action observation. Methods. The right pSTS was identified in the native cortical surface of 24 participants using a traditional biological motion localizer. The bilateral IFG, IPL, and an anterior STS region were identified as those parcels that were functionally connected to the right pSTS seed. Functional connectivity was computed on trial-based beta estimates acquired when observers viewed action vignettes (jumping up to reach a high box, crouching down to reach a low box) under two attention instructions: attend to action or attend to goal. Paired t-tests identified those parcels for which functional connectivity was modulated by the attention instruction and functional networks derived from the Yeo et al (2011) 7-network system were assigned. Results. The participant's attentional states modulated pSTS connectivity to the right IFG and anterior right STS, predominantly in the frontal control and default mode networks, such that connectivity was strengthened when participants attended to action as compared to attending to action goals.

These increases in connectivity were decoupled from the univariate response, which was stronger when attending to goal rather than action. There was no attentional modulation on pSTS connectivity to the IPL. Conclusion. Functional connectivity and brain mapping reveal distinct properties of the AON. Attention directed to actions strengthens the connectivity in a right-lateralized system that excludes the IPL. Thus the viewer's attentive state during action observation has the potential to restructure the coupling of nodes within the AON network.

Disclosures: X. Zhou: None. **D.A. Stehr:** None. **J.A. Pyles:** None. **E. Grossman:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.03

Topic: H.02. Perception and Imagery

Support: NIH Grant 5R01MH111439-02

Title: Human intracranial recordings of neural responses to visual dynamics in movies

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Abstract: Our visual experience in daily life is dominated by movement -- movement of objects in the world, movements of the eyes and changing perspective due to head and body movement. Neural processing of such dynamic visual input has been studied in movie watching experiments. Past studies have focused either on visual motion, eye movements or scene cuts. However, these features have not been analyzed in combination, which seems imperative, as they clearly interact. Eye movements are guided by moving objects, and scene cuts elicit saccades. Neural visual processing, in turn, is modulated by saccades. Here we focus on the interaction of these dominant visual dynamics in movies. We explore intracranial recordings (4477 electrodes) from 19 patients who passively watched up to 50 minutes of videos. Intracranial recordings capture neural responses at the rapid time scale of these visual dynamics. They also provide good spatial resolution to identify distinct responses in different brain areas. Linear neural responses were estimated for the local field potentials (LFP) as well as broadband high-frequency amplitude (BHA, 70-150 Hz). Only a few electrodes showed significant responses to mean optical flow, a measure of overall motion in the video. However, we found strong responses to scene cuts and saccades, lasting up to 1000 ms and extending far beyond traditional visual processing areas of the brain. Among saccades, we found stronger responses immediately following scene cuts. In

the parietal, temporal and frontal cortex we found long lasting BHA and LFP responses beyond 1000 ms for saccades after scene cuts, but not spontaneous saccades. In contrast, in the occipital cortex, saccades after scene cuts and spontaneous saccades show similar increases in BHA peaking about 200ms after saccade onset. Among scene cuts, we found stronger responses for those cuts that mark a transition in the narrative of the video. Importantly, higher level cortical areas, the parietal, temporal and frontal lobes, were more selective in responding to these narrative cuts. In contrast, occipital electrodes were not selective for the type of scene cut. The BHA responses were mostly characterized by an increase of neural activity 200ms-800ms following a scene cut, regardless of brain location. Taken together we conclude that novelty in the visual input is the dominant driver of neural activity. Specifically, meaningful semantic content leads to stronger and longer lasting responses that reach higher cortical processing stages.

Disclosures: M. Nentwich: None. **M. Leszczynski:** None. **B.E. Russ:** None. **S. Bickel:** None. **N. Markowitz:** None. **A.D. Mehta:** None. **C.E. Schroeder:** None. **L.C. Parra:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.04

Topic: H.02. Perception and Imagery

Support: Hertz Fellowship
NIH T32GM008208
NIH R01MH107797
NSF 1734907
NIH R21 EY030297

Title: A week in the life of the human connectome: stable homeostasis and characteristic excursions

Authors: *M. WANG[1,3], M. WARD[4], M. G'SELL[2], M. RICHARDSON[5,4], A. S. GHUMAN[4,1]; [1]Program of Neural Computation, [2]Statistics, Carnegie Mellon Univ., Pittsburgh, PA; [3]Med. Scientist Training Program, [4]Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; [5]Neurosurg., Massachusetts Gen. Hosp., Boston, MA

Abstract: During the course of a day, our brains must accomplish a wide range of tasks and demonstrate a remarkable amount of flexibility despite their anatomic stability. How do ecologically valid brain states balance the tension between these demands of flexibility and stability? To answer this question, we explored how the human functional connectome changes using continuous intracranial electroencephalography recordings in six epilepsy patients while

they go about their day: eating, talking with visitors, reading, etc. over the course of a week. By tracking how the coherence between all pairs of the 100-120 electrodes implanted in each patient changes over each five second window during the entire week, we were able to use unsupervised autoregressive methods to identify the prevalent dynamic patterns of connectivity. Two major patterns emerged. First, brain networks had a stable baseline state that the brain would consistently return to after individual subnetworks took excursions of various types throughout the day. This stable state was similar across all our subjects, consisting of elevated lower beta coherence and decreased theta and gamma coherence. Our second finding was that there was a discrete set of probable ways to leave this baseline state. Different sub-networks of the brain were not activated or inactivated randomly to each other: they formed a specific set of patterns of which networks could be activated together over which frequencies. These patterns were well-preserved from day to day: if one network's beta activation were linked to another network's gamma inactivation in one day, the same would generally hold true in other days. Additionally, the length of the excursion (e.g. the autocorrelation of each dynamic pattern) was consistent from day-to-day. These patterns show that after perturbations, the brain's functional networks are pulled to return within a stable baseline dynamic range that is similar from person-to-person, which may represent an optimal homeostatic state for the functional connectome. Excursions from this state occur frequently, presumably to accomplish tasks such as sleep or heightened activity, but the excursions are always marked by a return back to homeostasis. The day to day consistency of the largest excursions from homeostasis may indicate some underlying anatomic or energy limitation that forces departures from homeostasis to follow characteristic trajectories. Taken together, these results suggest a homeostasis-like mechanism by which the functional connectome achieves stability while allowing for neurocognitive flexibility.

Disclosures: M. Wang: None. **M. Ward:** None. **M. G'sell:** None. **M. Richardson:** None. **A.S. Ghuman:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.05

Topic: H.02. Perception and Imagery

Title: A novel experimental paradigm to investigate awareness of action

Authors: *D. S. JIN[1], M. S. KHURANA[2], S. L. AERTS[1], E. SIFF[1], I. FU[2], S. I. KRONEMER[1], K. L. CHRISTISON-LAGAY[3], J. LI[2], A. KUMAR[2], N. C. FREEDMAN[2], G. P. CHANDRASEKARAN[2], S. TINAZ[3], H. BLUMENFELD[3];
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Abstract: Loss of awareness of action is a common phenomenon in which complex, overlearned motor actions are performed but later are not reportable as aware experiences. Although previous research has investigated the generation of volitional actions and the awareness of intention to act, to date there have been no studies which isolate individual actions performed with and without awareness. Here, we developed a novel behavioral paradigm which distinguishes between actions with and without awareness. Our task uses aspects of inattentional blindness to achieve these outcomes and is readily adapted to EEG, pupillometry and other neurophysiological measures. Our experimental paradigm centered around repeated presentations of the classic board game Rush Hour, where a target block is moved to a key location by clearing away blocks which impede the path of movement. Subjects are explicitly tasked with recalling details from a background video constantly playing behind the game to incorporate inattentional blindness. Subjects recount details in a three-minute free-recall period after each ten-minute run. Randomly, subjects may be asked a two-part quiz: 1. A four-option multiple choice question where subjects choose their most recent move, and 2: A sliding scale on which subjects indicate their confidence in their choice. To learn the paradigm, subjects first perform a training day without quizzes. Then, subjects participate in two testing days with the quizzes. Subjects' self-assessed confidence was deemed "high" if it was in the top quartile of slider positions, or "low" if it was in the bottom quartile. Moves where questions were answered correctly with high confidence were rated as "aware". Moves where questions were answered incorrectly with low confidence were rated as "unaware". Our method produced high rates of agreement between answer result and confidence scores. In the top quartile of subject confidence, 93.2% of answers were correct. In the bottom quartile of subject confidence, only 28.7% of answers were correct, close to chance (25%) performance. In addition, our paradigm produced sufficient numbers of "aware" (mean 30.7) and "unaware" (mean 22.9) moves per subject across the two testing days for use with EEG and other neurophysiological measures. In conclusion, our task provides a means of distinguishing discrete actions performed with and without awareness, with high levels of validation and sufficient sample size for analysis. These discrete actions will be useful in determining the spatiotemporal dynamics of pre-action anticipatory voluntary motor signals and post-action perceptual signals in further testing with EEG and neuroimaging methods.

Disclosures: D.S. Jin: None. **M.S. Khurana:** None. **S.L. Aerts:** None. **E. Siff:** None. **I. Fu:** None. **S.I. Kronemer:** None. **K.L. Christison-Lagay:** None. **J. Li:** None. **A. Kumar:** None. **N.C. Freedman:** None. **G.P. Chandrasekaran:** None. **S. Tinaz:** None. **H. Blumenfeld:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.06

Topic: H.02. Perception and Imagery

Title: A novel visual perception no-report paradigm reveals early and broad responses from whole brain fMRI and high-density scalp EEG

Authors: *S. KRONEMER[1], T. XIN[1], J. DING[1], M. AKSEN[1], J. RYU[1], J. DING[1], J. PRINCE[1], H. KWON[1], A. KHALAF[1], S. FORMAN[1], M. KHOSLA[1], D. JIN[1], K. WANG[1], K. CHEN[1], C. HU[1], A. AGARWAL[1], E. SABERSKI[1], S. A. WAFA[1], O. MORGAN[1], W. HERMAN[1], J. WU[1], T. CONSTABLE[1], M. PITTS[2], M. CROWLEY[1], H. BLUMENFELD[1]; [1]Yale Univ., New Haven, CT; [2]Reed Col., Portland, OR

Abstract: Identifying the core neural mechanisms of consciousness is confounded by post-perceptual processing. The development of so-called no-report paradigms aims to relieve the engagement of cognitive processes that can be confused for perception-linked signals. Recent findings from no-report paradigms suggest late electrophysiology and higher-order association cortices are muted in the absence of overt report. However, these findings are nascent, there are competing results and interpretations in the literature, and little work has been done to investigate the subcortical dynamics in conscious perception. The current investigation utilizes a novel visual, perceptual-threshold report and no-report paradigm where overt report is replaced by machine learning based predictions of stimuli perception determined from eye tracking and pupillometry dynamics. Concurrent fMRI and high-density scalp EEG recordings allow access to both high spatial and temporal resolution dynamics of the healthy, adult brain physiology in processing visual conscious perception. In the overt report condition, there are early (<4s) blood-oxygen-level-dependent (BOLD) responses that distinguish perceived and not perceived stimuli in detection, arousal, and salience (DAS) network sites, including the primary visual cortex, dorsal lateral prefrontal cortex, anterior cingulate cortex, thalamus, and midbrain. At later times (>5s), widespread and sustained BOLD increases in the posterior parietal and prefrontal cortices and decreases in the default mode network (DMN) are found. Scalp EEG responses in the report condition show four prominent event-related potentials: N100, visual awareness negativity (VAN), N2, P2, and P3. Only the N100 is shared between the perceived and not perceived stimuli. In the no-report condition, perceived versus not perceived early BOLD signal dynamics are again found in DAS regions, including midbrain, thalamus, frontal, and parietal cortices. However, late responding BOLD signals found in the report condition are muted or absent, including components of the task-positive network increases and DMN decreases. Scalp EEG responses in the no-report condition isolate the core perception-link ERP to the VAN, while the N100 is present for both perceived and not perceived stimuli and the late ERPs are muted or absent, including the P3. Together these results support an early and broad core network for visual conscious perception independent of report. Future studies should investigate the causal role of these cortical and subcortical regions.

Disclosures: S. Kronemer: None. **T. Xin:** None. **J. Ding:** None. **M. Aksen:** None. **J. Ryu:** None. **J. Ding:** None. **J. Prince:** None. **H. Kwon:** None. **A. Khalaf:** None. **S. Forman:** None. **M. Khosla:** None. **D. Jin:** None. **K. Wang:** None. **K. Chen:** None. **C. Hu:** None. **A. Agarwal:** None. **E. Saberski:** None. **S.A. Wafa:** None. **O. Morgan:** None. **W. Herman:**

None. **J. Wu:** None. **T. Constable:** None. **M. Pitts:** None. **M. Crowley:** None. **H. Blumenfeld:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.07

Topic: H.02. Perception and Imagery

Title: Investigating tactile conscious perception with a threshold task and intracranial EEG

Authors: *S. MAJUMDER[1], N. C. FREEDMAN[1], J. Z. DING[1], M. M. GUSSO[3], K. L. CHRISTISON-LAGAY[4], S. I. KRONEMER[5], D. S. JIN[6], M. KHURANA[1], I. FREEDMAN[5], T. BUI[5], K. WU[2], I. H. QURAISHI[1], A. SIVARAJU[1], E. DAMISAH[2], H. BLUMENFELD[4]; [1]Neurol., [2]Neurosurg., Yale Sch. of Med., New Haven, CT; [3]Programa de Pos-Graduacao em Tecnologia em Saude, Pontificia Univiersidade Catolica do Parana, Curitiba, Brazil; [4]Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT; [5]Neurol., [6]Yale Univ., New Haven, CT

Abstract: Recent studies have made significant progress towards understanding the spatiotemporal dynamics underlying consciousness. To this end, our lab has combined neural recordings with novel perceptual threshold tasks in visual and auditory domains, in which participants were played threshold-level stimuli and subsequently reported their percepts. These studies showed that while activity in Not Perceived trials is limited to early sensory areas, Perceived trials are characterized by increases in sensory areas and areas critical to detection, a transient pulse from subcortical regions, a wave of activity that spreads to association regions, and a monotonic decrease in the default mode network. To ascertain if this pattern of activity is shared across modalities, a tactile correlate of the tasks was used. A vibration, calibrated to the participant's 50% detection threshold, was delivered via vibrating tactor to one of a participant's non-thumb fingers; 14% of trials contained no vibration. After each trial, participants reported whether they (1) felt the vibration and (2) the location of the vibration; if they felt no vibration, they were instructed to guess randomly. In this study, our participants were patients undergoing intracranial electroencephalography (icEEG) for intractable epilepsy. Behavioral findings indicate that participants (n=5) perceived a vibration in 59.7% (SEM 3.2%) of vibration-present trials; the false positive rate was 9.5% (SEM 5.2%) for blank trials. When participants reported perception of the stimulus, they correctly indicated its location in 79% (SEM 3.2%) of trials; when they reported no perception, finger localization accuracy was 19% (SEM 2.3%; chance is 25%). icEEG recordings were analyzed for power in the high gamma frequency range (40-115 Hz), of its known relationship to local neural activity. In Not Perceived trials, post-stimulus activity was dominated by a spatiotemporally limited increase in gamma power in the somatosensory regions. However, in Perceived trials, the observed increases in gamma power in

somatosensory regions expanded outward to association regions and were accompanied by increases in regions associated with the salience network (such as anterior insula/frontal operculum) and decreases in regions associated with the default mode network (such as medial frontal cortex). These early results are in line with our previous findings in visual and auditory paradigms, indicating that there are shared mechanisms of conscious perception involving a broad network of cortical regions across sensory modalities.

Disclosures: S. Majumder: None. **N.C. Freedman:** None. **J.Z. Ding:** None. **M.M. Gusso:** None. **K.L. Christison-Lagay:** None. **S.I. Kronemer:** None. **D.S. Jin:** None. **M. Khurana:** None. **I. Freedman:** None. **T. Bui:** None. **K. Wu:** None. **I.H. Quraishi:** None. **A. Sivaraju:** None. **E. Damisah:** None. **H. Blumenfeld:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.08

Topic: H.02. Perception and Imagery

Title: Early neural activity changes associated with visual conscious perception

Authors: *A. KHALAF, S. I. KRONEMER, K. L. CHRISTISON-LAGAY, H. KWON, J. LI, H. BLUMENFELD;
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Abstract: The neural mechanisms of visual conscious perception have been investigated for decades. However, the spatiotemporal dynamics associated with the earliest neural responses following consciously perceived stimuli are still poorly understood. Using a dataset of intracranial EEG recordings, the current study aims to investigate the neural activity changes associated with the earliest stages of visual conscious perception. Subjects (N=10) completed a continuous performance task in which individual letters were presented in series and subjects were asked to press a button when they saw a target letter. Broadband gamma power (40-115Hz) dynamics were considered because of their known relationship to populational neural firing. In particular, target trials with button presses and non-target trials without button presses were filtered using a Butterworth band-pass filter to limit the frequency content of the trials to include only frequencies within the broadband gamma frequency range. Broadband gamma power was calculated by squaring the filtered signal amplitude of each trial. Afterwards, within each subject, gamma power time course of each trial was z-score transformed using the mean and standard deviation of the gamma power across all trials of that subject. To identify the statistically significant changes in post-stimulus z-scored gamma power compared to the baseline, we employed a spatiotemporal cluster-based permutation test. We analyzed changes that were common to both target and non-target stimuli as well as differences between targets and non-

targets.
Our results revealed early gamma power increases within 0-100 ms from stimulus onset, regardless of whether the stimulus was a target or a non-target, in bilateral visual processing cortex, medial temporal cortex, frontal eye fields, superior frontal, orbital frontal, inferior frontal, and left temporal/parietal cortex. At later times (>300 ms), increases in broad regions of left frontal, parietal, and temporal cortices were seen specifically for target stimuli suggesting that these regions are possibly a part of the cortical networks involved in responding to target stimuli (the right hand was used for the response). These findings suggest a broadly distributed cortical visual detection network including frontal, parietal, and temporal cortices is engaged within 100 ms after signal transduction from the retina.

Disclosures: A. Khalaf: None. **S.I. Kronemer:** None. **K.L. Christison-Lagay:** None. **H. Kwon:** None. **J. Li:** None. **H. Blumenfeld:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.09

Topic: H.02. Perception and Imagery

Title: Neural activity measured with intracranial EEG during an auditory conscious perception task

Authors: *K. L. CHRISTISON-LAGAY[1], N. C. FREEDMAN[3,6], C. MICEK[3,7], A. KHALAF[3], S. I. KRONEMER[3], M. M. GUSSO[3,8], L. KIM[4], J. Z. DING[3,9], S. FORMAN[2,10], M. AKSEN[3], A. ABDEL-ATY[4], H. KWON[3,11], N. MARKOWITZ[12], E. M. YEAGLE[12], S. BICKEL[13], E. ESPINAL[12], J. L. HERRERO[14], J. J. YOUNG[15], A. D. MEHTA[16], K. WU[5], J. L. GERRARD[17], D. D. SPENCER[18], E. C. DAMISAH[5], H. BLUMENFELD[2];
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Abstract: Although recent work has made significant headway in understanding the temporal and spatial dynamics of the neural mechanisms of conscious perception, much of that work has

focused on visual paradigms. To determine whether there are shared mechanisms for perceptual consciousness across sensory modalities, here we investigate the auditory domain. We developed an auditory task, in which target sounds (calibrated to a participant's 50% detection threshold) were embedded in white noise. Participants were subsequently prompted to report if they perceived the sound, and the sound's identity (to validate perception). When participants indicated they did not hear a sound, they were asked to randomly guess on the question about sound identity. This task was completed by patients undergoing intracranial electroencephalography (icEEG) for intractable epilepsy. We found that participants' (n=31) perception rate was 58.0% (2.0 % SEM) when a target was present; false positive rate was 8.5% (1.4% SEM) for blank trials. When participants indicated they heard a target, they correctly identified the sound in 89.2% (1.4% SEM) of trials; sound identification accuracy for unheard trials was 40.2% (2.0% SEM) (chance: 33%). Intracranial recordings from over 2,800 grey matter electrodes representing widespread cortical coverage have been analyzed for power in the high gamma range (40-115 Hz)—a frequency range that reflects local neural activity. We performed a series of cluster-based permutation analyses to identify clusters of significant activity across perceived and not perceived trial conditions. For not perceived trials, we find that significant activity is restricted to early auditory regions (and areas immediately adjacent to the Sylvian fissure); and that this activity appears at ~150 ms and lasts until ~800 ms post stimulus onset. Perceived trials also show activity in early auditory regions, but this is accompanied by activity in the right caudal middle frontal gyrus, and shortly thereafter by activity in non-auditory thalamus. This is followed by a wave of activity that sweeps through the higher auditory association regions and into parietal and frontal cortices, similar to the wave observed in our visual conscious perception paradigm. We also find a decrease below baseline activity in orbital frontal and rostral inferior frontal cortex beginning ~200 ms post stimulus onset, consistent with the 'switch' observed in our visual paradigms. In summary, we found a broad network of cortical and subcortical regions involved in auditory perception that are similar to the networks observed with vision, suggesting shared general mechanisms for conscious perception.

Disclosures: K.L. Christison-Lagay: None. **N.C. Freedman:** None. **C. Micek:** None. **A. Khalaf:** None. **S.I. Kronemer:** None. **M.M. Gusso:** None. **L. Kim:** None. **J.Z. Ding:** None. **S. Forman:** None. **M. Aksen:** None. **A. Abdel-Aty:** None. **H. Kwon:** None. **N. Markowitz:** None. **E.M. Yeagle:** None. **S. Bickel:** None. **E. Espinal:** None. **J.L. Herrero:** None. **J.J. Young:** None. **A.D. Mehta:** None. **K. Wu:** None. **J.L. Gerrard:** None. **D.D. Spencer:** None. **E.C. Damisah:** None. **H. Blumenfeld:** None.

Poster

P496. Human Perception I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P496.10

Topic: H.02. Perception and Imagery

Title: The thalamic awareness potential: subcortical neural mechanisms of visual conscious perception from fMRI and intracranial EEG

Authors: *Q. XIN[1], S. I. KRONEMER[1], J. DING[1], M. AKSEN[1], J. RYU[3], H. KWON[2], J. DING[2], J. S. PRINCE[2], A. KHALAF[2], S. FORMAN[3], M. KHOSLA[1], K. WANG[2], K. CHEN[2], C. HU[2], A. AGARWAL[2], E. SABERSKI[2], W. HERMAN[2], A. URBAN[4], M. RICHARDSON[5], J. WU[2], M. CROWLEY[2], T. R. CONSTABLE[2], H. BLUMENFELD[3];
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Abstract: Studying the neural mechanisms of consciousness is an important query in modern cognitive neuroscience with broad scientific and clinical implications. Most studies and debate for identifying the core consciousness network have focused on cortical physiology. However, subcortical signals have been largely neglected from consideration as a neural mechanism for transient conscious percepts. In this study, two cohorts were recruited to study the spatial and temporal subcortical signal dynamics linked to visual conscious perception. First, healthy, adult participants were recorded with whole-brain fMRI while completing a report-based visual perceptual-threshold task. Second, a cohort of people with epilepsy that were chronically implanted with depth electrodes targeting the centromedian nucleus of the interlaminar thalamus (Responsive Neurostimulator System, NeuroPace, Inc.) were recruited to complete the same visual perception task while simultaneously recording scalp EEG. Blood-oxygen-level-dependent (BOLD) responses between perceived and not perceived stimuli demonstrate widespread subcortical engagement, including in the midbrain, thalamus, striatum, and cerebellum. At later times, cortical networks were also found specifically responsive to perception, including BOLD increases in task positive and decreases in the default mode networks. These BOLD results are strengthened by the electrophysiological findings from thalamic depth channels that reveal a perception-linked thalamic awareness potential (TAP; peak latency=439±16ms after stimulus onset) that occurs after the cortical scalp event-related potentials N100 (109±5ms) and visual awareness negativity (227±9ms) but precedes the P3 (487±20ms). The transient dynamic of the TAP suggests that the thalamus may have a neuromodulatory role for discrete conscious events and potentially augmenting later cortical activity, for example, those related to the P3. Together, the BOLD and intracranial electrophysiological results support a role of subcortical regions in visual conscious perceptual events. Further work may help elucidate the possible mechanistic role of the TAP and other subcortical potentials by direct electrical stimulation while observing changes in perceptual threshold.

Disclosures: Q. Xin: None. **S.I. Kronemer:** None. **J. Ding:** None. **M. Aksen:** None. **J. Ryu:** None. **H. Kwon:** None. **J. Ding:** None. **J.S. Prince:** None. **A. Khalaf:** None. **S. Forman:** None. **M. Khosla:** None. **K. Wang:** None. **K. Chen:** None. **C. Hu:** None. **A. Agarwal:** None. **E. Saberski:** None. **W. Herman:** None. **A. Urban:** None. **M. Richardson:** None. **J. Wu:** None. **M. Crowley:** None. **T.R. Constable:** None. **H. Blumenfeld:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.01

Topic: E.04. Voluntary Movements

Support: FRM Foundation under grant No DBS20140930785
ANR-16-CE19-0005-01 (Brainspeak)
European Union's Horizon 2020 research and innovation program under grant No. 732032 (BrainCom)

Title: Neural ensemble dynamics and speech encoding in Broca's area

Authors: P. ROUSSEL[1], F. BOCQUELET[1], S. CHABARDÈS[2], ***B. YVERT**[1];
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Abstract: Broca's area is a key region of the cortical speech production and processing network, that has been widely investigated and shown to be activated during overt and covert speech production with functional imaging techniques. However, ensemble activity in Broca's area remains largely unexplored. In particular, the extent to which its dynamics share similarities between overt and covert speech production, and whether and how speech is encoded in this region at the level of a neural population is unknown. Here we addressed these two questions in a patient undergoing awake surgery for the removal of a tumor located below the cortical surface of Broca's area. A 96-channel intracortical Utah array was transiently inserted in Pars Triangularis and single unit activity could be recorded in this region while the patient read aloud or imagined pronouncing short sentences presented on a screen. First, we found that the overall dynamics of the population of recorded neurons was different between overt and covert speech as indicated by different trajectories in a low-dimensional space of neural firing. This was further assessed by the possibility to decode between overt, covert, and silence conditions well above chance level. Then, we further observed strong similarities in the firing patterns of small sets of individual neurons when a same sentence was overtly pronounced twice. Such similarity involved different sets of neurons depending on the sentence. This similarity of spiking for identical sentences was further quantified by computing for each neuron, the Viktor and Purpura distance between spike trains for all pairs of sentences. We found that identical sentences yielded higher spiking similarity than different sentences ($P<0.001$). Furthermore, when considering only different sentences, the spiking similarity was strongest for pairs of sentences with the lowest phonetic distance. Overall, these results indicate that Broca's area gets differently activated between overt and covert speech and that similarity in spiking dynamics occurring in this region may reflect similarity in the phonetic content of overtly produced speech.

Disclosures: P. Roussel: None. **F. Bocquelet:** None. **S. Chabardès:** None. **B. Yvert:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.02

Topic: E.04. Voluntary Movements

Title: Both tactile and visual feedback improve tongue motor ability in pointing the target on palatal surface, but tactile feedback led to stronger retention

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Abstract: Incomplete tongue motor control is a common yet challenging issue among individuals with neurotraumas and neurological disorders such as spinal cord injury (SCI), stroke, Parkinson's disease, and Alzheimer's disease. Incomplete tongue motor control may lead to serious limitations in accomplishing daily oral activities such as swallowing, speech, and respiration. There are also several tongue-controlled assistive technologies, developed for people with quadriplegia, which requires a lot of training and accurate tongue motor control to operate. To enhance the tongue motor control accuracy and overcome the problems and challenges associated with the incomplete tongue motor control, a few training methods have been developed and employed with multiple sensory modalities including visual, auditory, and tactile feedback. However, the effectiveness of each sensory modality and the combinations of them in tongue motor learning is still in question. The object of this study is to test the effectiveness of the tactile assistance on motor training and compare it with that of the visual assistance. To do so, eight healthy subjects performed the tongue pointing task, in which they were visually instructed to touch the target on the palate by their tongue tip as accurate as possible. Each subject wore a custom-made dental retainer with 12 electrodes distributed over the palatal area. For visual training, an array of LEDs corresponding to the electrode locations was shown on the computer screen. When one electrode was contacted, the corresponding LED would be turned on. The further away from the target, the brighter the color of the LED. For tactile training, a small protrusion was added onto the dental retainer as an indicator of the target, and electrical stimulation with different frequency was provided to the tongue. The further away from the target, the higher the stimulation frequency. A crossover design was selected to increase the statistical power and counterbalance the learning effect. One baseline session and three post-training sessions were conducted during three days. Experimental result showed that both visual and tactile feedback improved tongue motor control ability of pointing the target palatal location, and tactile training provided stronger retention than visual training. These results suggest that current sensory feedback was not enough for tongue pointing task, and therefore additional assistive sensory feedback improved the tongue pointing accuracy. The results also suggest that sensory assistance provided via corticobulbar pathway may contribute to stronger retention than sensory assistance provided via corticospinal pathway.

Disclosures: **B. Jiang:** A. Employment/Salary (full or part-time):; Texas A&M University. **J. Kim:** A. Employment/Salary (full or part-time):; Texas A&M University. **H. Park:** A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.03

Topic: E.04. Voluntary Movements

Support: NSF NCS 1835181
NSF GRFP DGE 1746045

Title: A gesture-based forward model for speech motor control

Authors: *J. VEILLETTE, H. NUSBAUM;
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Abstract: Vocal learning species are able coordinate the activity of hundreds of muscles to produce sound and swiftly correct movement errors despite intrinsic latencies between motor output and auditory feedback. This remarkable ability is thought to be facilitated by the maintenance of a forward model, which makes predictions about the current state of the body's periphery and the sensory consequences of movement to which feedback is compared. In the zebra finch, HVC neurons burst and interneurons are suppressed within milliseconds of critical points in the trajectory of the biophysical parameters that control the vibration of the glottis, which has been interpreted as a strikingly explicit neural implementation of a forward model. The present study demonstrates that gamma band power in the human electroencephalogram linearly tracks extremity in the trajectory of glottal control parameters with near-zero time-lag during the production of fluent speech. Given that humans and zebra finches do not share a common vocal learning ancestor, this finding suggests similar internal models of vocal tract physics have independently evolved in both species - pointing to a general principle of vocal motor control.

Disclosures: **J. Veillette:** None. **H. Nusbaum:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.04

Topic: E.04. Voluntary Movements

Support: NIH Grant NS098971

Title: Inhibition of ongoing speech production in the frontal cortex

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Abstract: An important capacity for normal speech is the ability to stop ongoing production quickly when required. This ability is crucial for maintaining smooth conversations and deficits in it are often indicative of speech disorders. Previous studies have investigated the neural control for canceling speech and other motor outputs before their onset. However, it is largely unknown how the brain controls speech termination when one has already started speaking. Here we studied this question by directly recording neural activity from the human frontal cortex while participants start and stop their speech following visual cues. We found increased high-gamma activity near the end of the production in the frontal cortex during cued stopping, which was not observed in the self-paced, natural finish of a sentence. Across single trials, a subset of frontal regions was activated according to the time of the stop cue or stop action. Activity in single electrodes and across populations distinguish whether stopping occurred before an entire word was finished. In addition, we ask how the neural process for stopping is related to concurrent articulatory control. We found that stop activity existed in largely separate regions from those in the sensorimotor cortex encoding articulator movements. Interestingly, there is increased functional connectivity between the area with stop activity and the area with articulator encoding during stopping, compared to that before the stop cue. Finally, we found that the area with stimulation-induced speech arrest overlapped with the area showing stop activity, suggesting that speech arrest may be caused by inhibition of speech production, not by disruption of motor activities. Together, these data provide evidence that neural activity in the frontal cortex may act as an inhibitory control signal that underlies the stopping of ongoing speech production.

Disclosures: L. Zhao: None. **A.B. Silva:** None. **E.F. Chang:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.05

Topic: E.04. Voluntary Movements

Support: F32 DC018200 Ruth L. Kirschstein postdoctoral fellowship from the National Institute on Deafness and Other Communication Disorders
Leon Levy Foundation Fellowship to M.O.
R01NS109367 from the National Institute of Neurological Disorders and Stroke to A.F.

Title: The Effects of Delayed Auditory Feedback on the Speech Production of Epilepsy and Parkinson's Disease Patients

Authors: *E. JENSEN[1,2,3], M. OZKER[2,3,1], M. POURFAR[2,4], L. PAN[1,2], A. MOGILNER[1,4], A. FLINKER[1,3,2];
[2]Neurol., [1]NYU Sch. of Med., New York, NY; [3]Comprehensive Epilepsy Ctr., New York, NY; [4]Neurosurg., NYU Langone Hlth., New York, NY

Abstract: Hearing one's self is a fundamental part of the ability to speak fluently and without error. The auditory system continuously monitors self-generated vocal sounds in order to detect vocalization errors, which allows for the adjustment of motor actions to achieve the intended vocalization. Meaning that when the auditory feedback becomes disrupted, the vocalization will become altered in order to compensate for this disruption (e.g. speaking loudly when listening to music over headphones). To study this auditory feedback control during speech production, we implemented a delayed auditory feedback (DAF) paradigm, which strongly disrupts speech fluency by causing stutter-like speech, characterized by syllable repetitions, prolonged words and longer pauses. We studied two different patient populations with a pre-existing conditions of Epilepsy (EP) and Parkinson's Disease (PD) who have undergone deep brain stimulation implantation. Subjects were visually presented with 3-syllable words and 8-word sentences in separate sessions. As they read aloud the presented stimuli, their voice was recorded by a microphone and played back to them with 0, 50, 100 or 200 millisecond delays through earphones. In regards to the PD participants, the sessions were completed once with an activated Deep Brain Stimulation (DBS) and once again with the DBS deactivated. We found that in both EP and PD patients the total articulation duration increased significantly with the amount of delay in both the sentence sessions and word sessions. We examined the data gathered during the sentence sessions to determine whether DAF prolonged words or pauses between the words within the sentence. Both subject groups showed a significant increase in pause durations and articulation durations with relation to delay (EP pauses: $F = 14.81$, $p = 0.00202$, EP words: $F = 13.97$, $p = 0.00249$, PD pauses: $F = 8.528$, $p = 0.0266$, PD words: $F = 7.521$, $p = 0.0336$). We found that PD patients prolonged the articulation duration significantly more than the EP patients, but not the pauses between the articulations. Interestingly, the DBS did not have a significant effect on the articulation duration or the pause durations for PD patients. Overall these results suggest that DAF slows down speech both by prolonging articulation duration and pause durations between the words. We showed that PD patients are more susceptible to the effects of the DAF than the EP patients. DBS for PD patients provides minimal contribution to clear and accurate speech production.

Disclosures: E. Jensen: None. **M. Ozker:** None. **M. Pourfar:** None. **L. Pan:** None. **A. Mogilner:** None. **A. Flinker:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.06

Topic: E.04. Voluntary Movements

Title: Voice power spectra of sustained vowel and fricative phonemic condition

Authors: *J. FORBES, S. DELMAS, Y. CHOI, B. YACOUBI KEYHANI, E. CHRISTOU; Univ. of Florida, gainesville, FL

Abstract: Phonation and articulation are the building blocks of human speech that are carried out by various frequencies for effective communication. Much of the work characterizing the frequency structure of speech focuses on frequencies from 100 to 5000 Hz where much of the phonetic information of speech resides. However, the successful production of human speech requires the interaction of various systems that are largely controlled by lower frequencies, particularly the motor system. To date, the characterization of human speech below 100 Hz remains largely unexplored. Here, we use an experimental paradigm that incorporates simple sustained production of three different vowels (/a/, /i/, /u/) and a voiceless fricative (/ʃ/). The three vowels are the most common vowels across the world's languages and the fricative represents a speech production that does not include phonation with the vocal folds as the source of the sound comes from turbulence within the vocal tract. We hypothesize that each of these speech conditions will exhibit a robust oscillatory structure below 60 Hz likely related to a central drive that extends through multiple systems. Five healthy adults (3 females) sustained a sound at a comfortable pitch and loudness for 5 seconds. They performed 10 trials producing 3 vowels (/a/, /i/, /u/) and a voiceless fricative (/ʃ/) with a rest of 10 seconds between each trial. For each condition, we analyzed the acoustic structure of the sound output by performing a power spectrum and quantified the absolute and relative power below 60 Hz. We found that each phonemic condition exhibited a similar acoustic structure below 60 Hz. Specifically, each condition had distinct peaks at 2-3 Hz, 4-5 Hz, 18-25 Hz, and 35-55 Hz. Moreover, this finding was consistent across all participants for both the absolute and relative power ($p > 0.3$). Whereas these peaks are present in natural and connected speech, they are often considered a result of language structure and arising from the opening and closing of the mandible during syllables. The presence of these common oscillations across different sustained vowels and a voiceless fricative (with no oscillations being generated vocal fold vibration) suggests that these oscillations may be the result of the central drive to the articulatory structure regardless of speech task. The results also provide a foundation for using these simple speech tasks to explore motor control across systems along with the effects of age and impairment.

Disclosures: J. Forbes: None. **S. Delmas:** None. **Y. Choi:** None. **B. Yacoubi Keyhani:** None. **E. Christou:** None.

Poster

P541. Speech Motor Control

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P541.07

Topic: E.04. Voluntary Movements

Title: Distribution analysis of the tongue motion amongst healthy population

Authors: *V. BRATLAND[1], G. SORESINI[1], D. BONDAVALLI[1], J. L. PATTON[2], H. ESMAILBEIGI[1];
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Abstract: Neural injuries affecting the tongue motion could have dire consequences on speech, swallowing, and breathing. Studies have shown that visualization of the tongue motion could assist in diagnosis of motor deficits impacting this organ. However, to date, all available visualization technologies(Imaging, Electropalatography, Electromagnetic Articulography, etc.) are not portable and require a clinical setting. Our team has developed a discreet Bluetooth-enabled intra-oral wearable that recognizes the tongue's contact inside the oral cavity and quantifies its motion. Hence for the first time enabling a user-friendly approach to studying tongue motion. In this preliminary work, we study healthy tongue motion to evaluate the presence of distinctive patterns. Ten healthy individuals were asked to randomly traverse their entire palate with the tip of their tongue (referred to as "free-exploration"), which motivated individuals to express the full tongue's motor capabilities. Distribution analysis of position, velocity, and acceleration for all individuals, both within and between subjects, suggest that healthy individuals exhibit an individualized motion pattern. This pattern is most distinguishable in the position probability distribution. Higher derivatives exhibit similarities amongst individuals, which could be attributed to physiological limitations. The current work represents an important initial step towards our larger goal of better understanding of both healthy and deficit-induced tongue motion characteristics.

Disclosures: V. Bratland: None. **G. Soresini:** None. **D. Bondavalli:** None. **J.L. Patton:** None. **H. Esmailbeigi:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.01

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
NIH NS114279
NYS SCIRB C32236GG
NYS SCIRB C33279GG
Stratton VA Medical Center

Title: The effect of tDCS on learning a simple motor skill: operantly conditioning the soleus H-reflex

Authors: *L. M. MCCANE[1,2], J. R. WOLPAW[1,3], S. E. D'ANDREA[4], A. K. THOMPSON[5]; [1]Natl. Ctr. For Adaptive Neurotechnologies, Albany, NY; [2]Univ. of Rhode Isalnd, Kingston, RI; [3]Sch. of Publ. Health, State Univ. of New York, Albany, NY; [4]Univ. of Rhode Island, Kingston, RI; [5]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: Transcranial direct current stimulation (tDCS) can modulate cortical activity. Excitatory tDCS (positive current into the brain) can enhance motor skill acquisition and retention (Brain Cogn 102: 1-12). The responsible mechanisms are not clear. Remarkably, little is known about tDCS's impact on the spinal cord. Spinal reflexes participate in our everyday movements, change throughout life, and can be modified by experience and injury (J Physiol 596(16): 3469-3491). People can change the size of a spinal reflex through operant conditioning (J Neurosci 29(18): 5784-5792). Acquisition of this simple motor skill (a larger or smaller spinal reflex) requires supraspinal drive and demonstrates task dependent adaption (TDA) (J Neurophysiol 87: 645-652). This study asks if excitatory tDCS affects the magnitude or consistency of operantly conditioned decrease in the human soleus H-reflex.
The study is approved by the MUSC IRB. Neurotypical participants receive Active (excitatory, 2mA, 0.06mA/cm^2, 30 min) or Sham tDCS over the contralateral leg region of primary motor cortex (M1) during soleus H-reflex down-conditioning (3 sessions/wk). Soleus H-reflexes are elicited by tibial nerve stimulation at the popliteal fossa (1ms sq cathodal pulse). Participants maintain soleus background activity in a defined range while comfortably standing. During 4 Baseline (BL) sessions, H-reflexes are simply recorded (control reflex trials, 3 blocks of 75 each). During 12 subsequent Conditioning sessions (CSs), participants practice reducing the reflex size from the mean BL value (reflex conditioning trials, 3 blocks of 75). Visual feedback about H-reflex size occurs after each reflex conditioning trial. H-reflex/M-wave recruitment curves and control reflexes (n=20) are collected at the start of each session. A 1-month follow-up (FU) session examines skill retention.
To date, 8 participants (4 women, 5 Active tDCS, Age 22-36) have completed the study. Final conditioned H-reflex sizes (mean of CSs 10-12 as % BL mean) were 58, 67, 90, 99, 215% for Active and 71,115,125% for Sham participants. Average TDAs for CSs 1-4, 5-8, 9-12 were (in % of means of the control trials): +10.9, -4.1, -6.7% and +0.5, +3.6, -6.4%, Active and Sham,

respectively. In 4 Active participants who completed the FU visit, the conditioned reflexes were 16, 57, 98, and 233% and TDA was -2.5, -2.6, -38, and +37%. In 2 Sham participants who completed the FU visit, the conditioned reflexes were 27 and 59% and TDAs were -6.5 and -8.5%. The study is ongoing. The results of this study may improve understanding of tDCS's impact on motor skill acquisition and guide its therapeutic use a neuromodulator.

Disclosures: L.M. McCane: None. **J.R. Wolpaw:** None. **S.E. D'Andrea:** None. **A.K. Thompson:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.02

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844 (Kautz)
NIGMS P20 GM109040 (Kautz)
NIBIB P41EB018783 (Wolpaw)
NINDS R01NS114279 (Thompson)
NINDS U44 NS114420 (Clements/Thompson/Wolpaw)
New York State Spinal Cord Injury Research Trust C33279GG (Wolpaw)
The Doscher Neurorehabilitation Research Program

Title: Operant down-conditioning of the soleus H-reflex in chronic stroke patients with spastic hemiparesis

Authors: *A. K. THOMPSON[1], C. R. GILL[1], W. FENG[3], R. L. SEGAL[2];
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Abstract: Introduction: Through operant conditioning, spinal reflex behaviors can be changed. Previous studies in rats indicate that the sensorimotor cortex and corticospinal tract are essential in producing and maintaining reflex changes induced through conditioning. In individuals with incomplete spinal cord injury (SCI), an operant down-conditioning protocol can decrease the soleus H-reflex size and improve walking (J Neurosci 2013;33:2365-2375; J Physiol 2021;599:2453-2469), suggesting that partially preserved spinal cord can support conditioning-induced plasticity and benefit from it. This study aimed to exam whether down-conditioning can decrease the soleus H-reflex in people with supraspinal injury, cortical or subcortical stroke.Methods: Operant down-conditioning was applied to the soleus H-reflex in 12 participants (37-76 years old, 1.5-11.5 years post-stroke) with spastic hemiparesis due to chronic stroke. Each participant completed 6 baseline and 30 conditioning sessions over 12 weeks. In each baseline

session, 225 control H-reflexes were elicited without any feedback on H-reflex size. In each conditioning session, 225 conditioned H-reflexes were elicited while the participant was asked to decrease H-reflex size and was given visual feedback as to whether the resulting H-reflex was smaller than a criterion value. Before and after conditioning, 10-m walking speed was measured. Results and Discussion: In 6 out of 12 study participants with stroke, H-reflex size decreased significantly over the course of conditioning ($p<0.05$) and their walking speed was significantly increased (+0.14m/s) after conditioning. Their final reflex size (70±18% baseline, mean±SD) was similar to that in people with chronic SCI (69±27%, J Neurosci 2013;33:2365-2375), yet its composition and development differed from that in SCI; it consisted of robust within-session conditioning-task-dependent change (-12%) and slow and limited across-session long-term change (-18%), in contrast to 7% within-session change and -14% long-term change in SCI. In other 6 participants with stroke, H-reflex did not become smaller (final size: 104±8% consisted of -14% within-session change and +18% long-term change) and the walking speed did not change (+0.00m/s). Differences in the extent of within-session task-dependent adaptation, the rate of long-term plasticity development, and the conditioning success rate across populations may reflect a potentially critical role of supraspinal (i.e., cortical) activity in producing the spinal cord long-term plasticity.

Disclosures: A.K. Thompson: None. **C.R. Gill:** None. **W. Feng:** None. **R.L. Segal:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.03

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844-01 (Kautz)
NINDS NS114279
The Doscher Neurorehabilitation Research Program

Title: Effects of dry needling of the medial gastrocnemius on ankle range of motion and spinal reflexes in people with and without chronic stroke

Authors: *G. SEIF[1], A. PHIPPS[1], M. MCLEOD[1], J. DONNELLY[2], C. FERNANDEZ-DE-LAS-PENAS[3], A. ZULOAGA[1], R. A. MCLAUGHLIN[1], B. DELLENBACH[1], A. THOMPSON[1];
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Abstract: Introduction: To define the clinical utility of dry needling (DN), it is essential to understand its neurophysiological impact on CNS pathways involved in sensorimotor function.

Mechanical actions of DN are as follows: DN that targets trigger points (TrP) can restore muscle fiber length by disrupting dysfunctional motor end plates and stretch the shortened sarcomeres. DN stimulates nociceptors. DN can activate serotonergic and noradrenergic systems and influence the impact of sensory inputs in the spinal dorsal horn. DN increases the local muscle blood flow and oxygen saturation. While the understanding of mechanisms of actions continues to improve, to date, how DN affects spinal and supraspinal somatosensory processing is not well understood. Thus, the purpose of this study was to examine neurophysiological effects of DN on spinal reflexes in people with and without stroke.
Methods: Before, 0, 90 min, and 72 hours after a single bout of DN of a TrP in the medial gastrocnemius (MG), the triceps surae (i.e., soleus, MG, and lateral gastrocnemius (LG)) H-reflexes and reciprocal inhibition were examined; and the perceptual threshold (PT) of cutaneous distal tibial nerve (DTN) and superficial peroneal nerve (SP) stimulation were determined. All measurements were made while the participant stood and maintained natural standing posture and soleus muscle activity. Ankle dorsiflexion (DF) range of motion (ROM) was also measured.
Participants: 14 adults ages 22-57 years old without stroke and 11 adults ages 35-70 with clinical spasticity due to stroke. **Results:** In people without stroke, at 72 hours after DN the MG H-reflex amplitude was decreased (-13% from pre DN, p=0.003 by paired t-test); reciprocal inhibition of the soleus was increased (+68%, p=0.019); PT for the DTN stimulation was decreased (-19%, p=0.009); and ankle DF ROM was increased (+4 deg, p=0.003). In people with stroke, the ankle DF ROM was increased at 0 (+4.6 deg, p=0.000), 90 min (+3.1 deg, p=0.024), and 72 hours post (+4.9 deg, p=0.001), while reflex changes were inconsistent across individuals.
Discussion: In persons without stroke, DN produces neurophysiological effects in multiple spinal pathways that are clearly present at 72 hours post. In persons with stroke, DN increases DF ROM; yet the current sample size is not sufficient to elucidate its impact on spinal neurophysiology. To overcome the neurophysiological heterogeneity of people with stroke, further studies are currently underway. Understanding the neurophysiological effects of DN will help clinicians determine optimal DN applications, including the timing of post-DN intervention implementation.

Disclosures: G. Seif: None. **A. Phipps:** None. **M. Mcleod:** None. **J. Donnelly:** None. **C. Fernandez-de-las-Penas:** None. **A. Zuloaga:** None. **R.A. McLaughlin:** None. **B. Dellenbach:** None. **A. Thompson:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.04

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844-01 (Kautz)
NIBIB 1P41EB018783 (Wolpaw)
NINDS NS114279 (Thompson)
The Doscher Neurorehabilitation Research Program
South Carolina Spinal Cord Injury Research Fund (SCIRF#2014 R01, #2019 PD-01)

Title: Altered task-dependent modulation of cutaneous reflexes in people with chronic incomplete spinal cord injury

Authors: *A. M. PHIPPS, A. K. THOMPSON;
Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: In individuals without neurological injuries, cutaneous (CUT) reflexes to non-noxious stimuli are task-dependently modulated (Brain Res. 1993:613:230-238). Currently, CUT reflexes in ambulatory individuals with spinal cord injury (SCI) are not well understood. The purpose of this study was to examine CUT reflexes in the triceps surae during standing and walking in SCI. Eight individuals with chronic incomplete SCI and eleven age-matched controls free from injury participated in this study. CUT reflexes were elicited by stimulating the sural (SR), superficial peroneal (SP), or distal tibial nerve (TN) near the ankle of the clinically more spastic leg in SCI and the left leg in controls. For each nerve stimulation, a 5 x 1-ms pulse train (200 Hz) at ≈ 1.7 x radiating threshold was delivered while the participant stood upright and while they walked at his/her comfortable speed on a treadmill for 4.5-6 minutes. Electromyography was recorded from the triceps surae (soleus (SOL) and medial (MG) and lateral gastrocnemius (LG)) ipsilateral to the stimulation site. Short (50-80 ms post-stimulus, SLR) and medium (80-120 ms, MLR) latency CUT reflexes in the triceps surae were quantified in standing and during the early stance phase of walking and normalized to the maximum M-wave (M-max) elicited by single pulse posterior tibial nerve stimulation during standing.
In the SCI group, following SR stimulation, the SOL SLR showed greater suppression in walking (-0.58% M-max) compared to standing (-0.24%) ($p = 0.022$ by paired t-test). However, in controls, the SLR was different following SP stimulation, showing a reversal from excitation in standing (0.43, 0.14, and 0.05%) to suppression during walking (-0.62, -0.49, and -0.21%) for the SOL ($p = 0.02$), MG ($p = 0.035$), and LG ($p = 0.03$), respectively. SCI did exhibit reflex reversal in the MG MLR following SP stimulation (0.01% standing vs. -0.21% walking, $p = 0.042$). A reversal of the MLR was also seen in the control group following SP stimulation, but in the SOL (0.36% standing vs. -0.83% walking, $p < 0.001$). In controls, following SR stimulation, both the SOL and MG showed greater suppression in walking compared to standing ($p = 0.028$ and $p = 0.014$, respectively).
CUT reflexes are task-dependently modulated in individuals with SCI, exhibiting both magnitude differences (i.e. greater suppression) and reflex reversal. Present, yet altered CUT reflexes in SCI, may serve as unique therapeutic training targets for guiding beneficial plasticity through neuromodulation interventions for rehabilitation in people with SCI.

Disclosures: A.M. Phipps: None. **A.K. Thompson:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.05

Topic: E.06. Posture and Gait

Support: NIH NINDS R01NS114279 (Thompson)
NICHD P2C HD086844 (Kautz)
NIBIB P41EB018783 (Wolpaw)
The Doscher Neurorehabilitation Research Program

Title: The effects of speed and weight on the soleus and lateral gastrocnemius muscle activity during walking

Authors: *B. A. POULIOT, A. K. THOMPSON;
Hlth. Sci. and Res., Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: Introduction: Previous studies have shown differential contribution of Ia, II, and Ib afferents to the stance phase soleus activity in human locomotion (J Physiol 2000;523:817-827; J Neurophysiol 2005;93:167-177). To further our understanding of afferent contribution to locomotor control, here we investigated the soleus and lateral gastrocnemius (LG) H-reflexes and muscle activity at different walking speeds with different weight bearing conditions. Methods: To ensure the robust impact of fixed weight and speed conditions, all participants were young women of similar physical size (27±6 yr, 163±4 cm, 65±9 kg) with no history of neurological or orthopedic injury. Each participant walked on the treadmill at 1.0 and 1.5 m/s with or without an additional 20.4 kg, which was worn as a set of weighted belt and vest, either on the front or back of the body. During each of the six (1.0 and 1.5 m/s speeds x none, front, and back weight conditions) ≈10-minute treadmill walking bouts, the soleus and LG locomotor EMG and H-reflexes, ankle, knee, and hip joint motion, and ground reaction force (GRF) were measured.Results: Overall, speed and weight affected the step cycle duration (≈230 ms difference across conditions) but produced only minor effects on hip and knee joint motion and their peak flexion/extension timings (i.e., all effects remained within 10° or 4% gait cycle range). Speed clearly affected ankle motion; late stance dorsiflexion (DF) was ≈5° less and end stance plantarflexion was ≈10° more and they reached the peak angles 4-5% earlier in gait cycle with 1.5 m/s than 1.0 m/s. Corresponding to the impact of speed on ankle joint motion, the late stance EMG amplitude with 1.5 m/s was nearly 200% of that with 1.0 m/s (178-193% for soleus and 195-214% for LG). The late stance H-reflexes were also larger with 1.5 m/s than with 1.0 m/s but to a lesser extent (+12-30%). Further analyses of mid-late stance ankle joint motion and GRF revealed slower DF (-5°/s) and 2-3x higher rate of force development (RFD) with 1.5 m/s than with 1.0 m/s, suggesting the occurrence of enhanced loading with 1.5 m/s during this phase.

Added weight appeared to enhance DF speed reduction and RFD increase. Discussion: Walking speed has significant impact on plantarflexor activity and ankle joint motion in the mid-late stance phase. Small increases in H-reflex amplitude relative to background EMG, together with robust increases in RFD and reductions in DF, observed during 1.5 m/s walking support a possibility that load-sensitive afferents play a major role in increasing plantarflexor activity in response to increasing gait speed. To better assess the effects of added weight, further analyses are currently underway.

Disclosures: B.A. Pouliot: None. **A.K. Thompson:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.06

Topic: E.06. Posture and Gait

Support: NSF Grant 2015317

Title: Investigating the role of low level reinforcement reflex loops in insect locomotion

Authors: *C. GOLDSMITH[1], R. D. QUINN[2], N. SZCZECINSKI[1];
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Abstract: Insects have been identified as highly capable walkers, but how the insect nervous system controls locomotion is not presently understood. In particular, it is unknown what precise mechanisms exist in the ventral nerve cord (VNC) to control locomotion, and the information passed between this ``lower level" and the ``upper level" areas of the head ganglia to facilitate this control is unclear. In this work, we seek to explore these questions by investigating how systems traditionally described as ``positive feedback" may give rise to stepping in the VNC. We focus on the `reflex reversal' of insect joints between a resistance reflex (RR) and an active reaction (AR) in response to joint flexion, as well as the excitement of populations of descending dorsal median unpaired (desDUM) neurons from limb strain as our primary reflex loops. We discuss the development of a neuromechanical model of the stick insect (*Carausius morosus*) femur-tibia (FTi) and coxa-trochanter (CTr) joint control networks "in-the-loop" with a physical robotic limb. We based our network on mappings of the non-spiking interneuron (NSI) joint control network for FTi joint, extrapolated network connectivity based on known biological responses, and previously developed morphological mechanisms to produce sideways stepping. To determine the mechanisms behind reflex reversal, we selectively inhibited the network's sensory afferents of the femoral chordotonal organ (fCO) and observed the reflexes of the simulated joint to a ramp-and-hold flexion stimulus. We find that inhibiting the network's flexion

position and velocity afferents enacts a reflex reversal in the FTi joint. We also explored the results of the full network sustaining steady locomotion on our test limb. Our findings suggest that the reflex reversal and limb strain reinforcement mechanisms are both necessary but individually insufficient to produce and maintain rhythmic stepping in the limb, which can be initiated or halted by brief, transient descending signals. Removing portions of this feedback loop or creating a large enough disruption can halt stepping independent of the higher-level centers. We conclude by discussing why the nervous system might control joint behavior in this manner, as well as how to apply these findings to generalized nervous system understanding and improved robotic control.

Disclosures: C. Goldsmith: None. **R.D. Quinn:** None. **N. Szczecinski:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.07

Topic: E.06. Posture and Gait

Support: NIH, Grant K01 HD100588 to MAL

Title: Differential effect of quadriceps muscle and femoral nerve stimulation onto Soleus H-reflex.

Authors: *C. CUADRA[1,2], S. L. WOLF[1], M. A. LYLE[1];
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Abstract: Intermuscular feedback from proprioceptors (e.g. muscle spindle, Golgi tendon organ [GTO]) has an important role in motor coordination, but the unique action of individual circuits is not well understood. A better understanding would help clarify their functional role during movement and how alterations caused by neuropathology contribute to motor impairments. Traditionally, nerve stimulation has been used to evaluate intermuscular neural effects, but this technique activates sensorimotor axons based on diameter, which biases spindle feedback. We have recently found that quadriceps muscle stimulation evoked twitch contractions result in intermuscular inhibition with minimal excitation of spindle axons compared to nerve stimulation. We can use this approach to study intermuscular feedback in patient populations (e.g., stroke), but our prior study required a steady ~20% max soleus contraction that could be challenging for patients. In this study, we evaluated the influence of intermuscular feedback from quadriceps onto Soleus H-reflex (Sol-HR) generated by pairing stimulation of tibial nerve (TNS) with femoral nerve and quadriceps muscle stimulation (FNS, QMS). We hypothesized that FNS, but not QMS, will generate an increase in Sol-HR when spindle afferent volleys from TNS and

FNS/QS are timed to reach the spinal cord concurrently (excitation periods). We also predicted that inhibition of Sol-HR produced by FNS and QMS will be similar if inhibitory volleys (GTO/Recurrent inhibition) from FNS/QMS are timed to arrive onto Sol motoneurons concurrently with TNS volleys (inhibitory periods).13 participants were seated on a dynamometer with ankle fixed in an ankle immobilizer. Participants targeted 6-10% of their Sol MVIC during H-reflex recruitment and testing trials. Sol-HR size was compared when recorded with TNS only (test reflex, ~40-60% Sol-HR max) and when TNS was paired with either FNS or QMS (conditioned reflex). Test and conditioning stimuli were randomly alternated over ~20 trials for each. FNS and QMS intensities (~1.5 vs ~2x motor threshold) were selected so that knee extension torque was matched between both stimulation conditions. As predicted, FNS resulted in significantly greater excitation (larger Sol-HR) compared to QMS during excitation periods. QMS and FNS produced similar levels of inhibition for each of the inhibitory periods. Our results suggest that muscle twitch contractions may be useful to bias intermuscular inhibitory pathways with reduced influence from spindle feedback. In addition to force dependent GTOs, inhibitory feedback could arise from recurrent inhibitory pathways.

Disclosures: C. Cuadra: None. **S.L. Wolf:** None. **M.A. Lyle:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.08

Topic: E.06. Posture and Gait

Support: Eunice Kennedy Shriver National Institute of Child Health & Human Development of the NIH, Award Number R03HD094583
Eunice Kennedy Shriver National Institute of Child Health & Human Development of the NIH, Award Number 1F30HD103318-01A1

Title: The effect of task complexity on h-reflex gain in children with spastic cerebral palsy

Authors: *B. C. CONNER[1], Z. F. LERNER[2,1];
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Abstract: Cerebral palsy (CP) is a pediatric onset movement disorder caused by injury to the developing brain. A common feature in CP is spasticity, which is characterized by exaggerated stretch reflexes that can lead to inefficient movement patterns. Understanding the underlying mechanisms of these exaggerated reflexes may be used to inform present and future interventions. In healthy individuals, it has been observed that the soleus H-reflex gain, a measure of stretch reflex excitability, decreases with increased task complexity. It was

hypothesized that this behavior reflects a supraspinal mechanism for preserving the motor pool for the task at hand. The purpose of this exploratory study was to determine the effect of task complexity on stretch reflex excitability in CP. Six children with CP (12 to 18 years, Gross Motor Function Classification System levels I - III, spastic hemiplegic and diplegic distributions) were recruited for this study. Surface electromyography (EMG) sensors were placed over the soleus of the non-dominant leg of each participant for measuring reflex responses and muscle activity. Stimulating electrodes were placed in the popliteal fossa of the participant's non-dominant leg for stimulation of the posterior tibial nerve. Stimulations were 1 ms square-wave pulses ranging from 1 - 30 mA. Reflex responses were evoked at a low level of stimulation and progressively increased until a visible M-wave was present. Reflex responses were then recorded for participants under two conditions: 1) standing on both legs (less complex condition), and 2) standing on the non-dominant leg (more complex condition). Fifteen reflexes were recorded for each condition where the associated M-waves were within ± 10% of one another to ensure a consistent stimulus strength. Background EMG (bEMG) activity was measured 50 ms before each respective stimulation. H-reflex amplitudes were measured as the peak-to-peak magnitude of the raw EMG signal. H-reflex gain was calculated for each condition as the change in H-reflex amplitude relative to the change in mean bEMG activity within a condition. Four out of six participants had an increase in H-reflex gain (31 ± 23%) with increased task complexity, representing an atypical response of increased stretch reflex excitability. The two participants who had a typical response of decreased H-reflex gain (-21 ± 7%) had undergone a selective dorsal rhizotomy (SDR). These findings offer insight into the effect of CP and SDR procedures on the supraspinal control of stretch reflexes with changes in task complexity, which could have important implications for rehabilitative interventions that seek to modulate these reflex responses.

Disclosures: B.C. Conner: None. **Z.F. Lerner:** None.

Poster

P570. Reflexes and Reflex Modulation I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P570.09

Topic: E.06. Posture and Gait

Support: NIH Grant T32 HD007418

Title: Stretch reflexes reflect volitional activity from several muscles about a joint during movement

Authors: *Z. VILLAMAR[1,3], D. LUDVIG[1,3], E. J. PERREAULT[1,3,2];
[1]Biomed. Engin., Northwestern Univ., Evanston, IL; [2]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Shirley Ryan AbilityLab, Chicago, IL

Abstract: The stretch reflex is a fundamental building block of motor function, modulating its sensitivity across tasks to augment volitional control. Reflex amplitudes increase with the background activity of the stretched muscle, known as gain scaling. Most studies of gain scaling have been performed in simple, isometric tasks without much co-contraction but amplitude modulation also exists during movement and tasks with co-contraction. Recent microneurography work shows that afferent activity from muscle spindles during movement represents the balance of activity within agonist and antagonist muscles acting about a joint. The objective of this study was to determine if the stretch reflex also reflects this balance of activity during movement and if this balance can account for the reported differences in reflex sensitivity between posture and movement.

Data were collected from 18 subjects. Each had their elbow fixed to a rotary motor via a fiberglass cast. We used surface electromyography to record activity from two elbow flexors and extensors during cyclic movements spanning 20 degrees. Subjects also performed isometric contractions at the mean posture of the movement trials; contraction levels spanned the range of activations measured during movement. The motor applied small pseudorandom perturbations during all trials to elicit stretch reflexes. Linear mixed effects models were used to determine how stretch reflex magnitude varied with agonist and antagonist muscle activity during movement compared to during posture.

Reflexes during movement were sensitive to agonist and antagonist background activity, as assessed by comparing models of reflex activity that considered background activity in the homonymous muscle to those that also considered agonists and antagonists. R^2 increased from 0.54 to 0.84 ($p = 0.004$) when all muscles were considered; activity in antagonists reduced reflex amplitude whereas agonists increased amplitude. Gain scaling from agonists was 75% lower in the flexor muscles during movement relative to posture and 89% lower in the extensor muscles (both $p = 0.0001$), indicating the balance of activity contributing to reflex modulation cannot account for all differences between posture and movement. In contrast, there were much smaller task-dependent changes in the effect of antagonist activity on reflex amplitude. Our results show how stretch reflexes at the elbow reflect the state of agonists and antagonists, and how gain scaling for all muscles changes between posture and movement. We identified two distinct mechanisms for modulating reflex sensitivity in the many tasks involving co-contraction and movement.

Disclosures: Z. Villamar: None. **D. Ludvig:** None. **E.J. Perreault:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.01

Topic: F.04. Stress and the Brain

Support: NIMH R01 MH115914
NIMH R01 MH115049

Title: Effects of early life adversity on stress-induced alcohol consumption and the VTA CRF system

Authors: *J. M. BRETON, K. G. BATH;
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Abstract: Experiencing early life adversity (ELA) increases the risk for developing substance use disorders later in life. Emerging evidence suggests that ELA alters reward and stress circuits, providing a potential mechanism underlying vulnerability to addiction. Animal models of ELA, such as the limited bedding and nesting (LBN) paradigm, may provide insights into the neurobiological factors that contribute to risk for drug seeking behavior. To date, there have been mixed findings as to whether LBN protects against or augments later-life drug seeking, with effects differing based on the class of drug, mode of administration, and sex of the subject. An important and less well understood factor that contributes to drug seeking is the state of the animal, including current or recent experience of stress. Indeed, a secondary stressor later in life may augment drug seeking in animals that have previously experienced ELA. However, little is known regarding how ELA might alter stress-induced drug seeking. Here, we tested whether exposure to an acute stress in the form of foot shocks altered ethanol consumption in control and LBN reared mice. Adult male and female mice in both groups underwent several weeks of voluntary ethanol consumption using a two-choice continuous access paradigm. Once stable drinking was established, mice were given a short series of foot shocks. Ethanol preference was temporarily altered after acute stress. Specifically, ethanol preference was reduced in LBN females for several days following the stressor before restabilizing to pre-shock levels. In addition, we assessed the effects of LBN on reward circuitry. We focused on the ventral tegmental area (VTA), a critical node for both stress and reward processing. The corticotropin releasing factor (CRF) system in the VTA has been previously linked with ethanol consumption and with stress-induced reinstatement of drug seeking. Here, we found that LBN increases CRF binding protein expression levels in the VTA, in both male and female mice. Additional work will be required to assess whether these changes in the VTA are functionally related to stress-induced changes in ethanol seeking. Overall, this work provides critical insight into the effects of ELA on stress-related behaviors, their circuits and the overlap between stress and the risk for addiction.

Disclosures: J.M. Breton: None. **K.G. Bath:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.02

Topic: F.04. Stress and the Brain

Title: Effects of Maternal Separation on Initial Reward to Alcohol in Mice: Influence of b-endorphin & Sex

Authors: M. R. SCOPANO[1], H. E. JONES[1], ***J. E. GRISEL**[2];
[1]Bucknell, LEWISBURG, PA; [2]Bucknell Univ., Lewisburg, PA

Abstract: Adverse childhood experiences, ACEs, affect 61% of the population and are associated with chronic health problems, mental illness, and substance misuse in adulthood (Center for Disease Control, 2020; Pilowsky et al., 2009). The response to stressful stimuli, such as an ACE, relies upon the opioid peptide β-endorphin which regulates the stress response and has been implicated in the risk for excessive drinking (Nentwig et al., 2019). Clinical research has demonstrated that initial sensitivity to the rewarding effects of alcohol is associated with stress sensitivity and predicts the risk of developing an alcohol use disorder, or alcoholism (e.g., Ray et. al, 2010, 2013). Here, we explore the effects of maternal separation on initial sensitivity to alcohol reward in mice, as measured using a single-exposure conditioned-place preference paradigm (SE-CPP) based on prior research (Grisel et al., 2014). Using female and male wildtype C57BL/6J (B6, n=22) mice and beta-endorphin-deficient mice (B6.129S2-Pomc tm1Low /J; KO, n=20), with sex as a biological variable, rodents were subjected to either maternal separation (MS, n= 3 KO♂**,** 4 KO♀, 7 B6♂, 6 B6♀) or no MS (CTL, n= 5 KO♂**,** 8 KO♀, 1 B6♂, 6 B6♀) for 3hr each day from postnatal days (PND) 5 to 18. They were weaned and group housed in standard conditions at PND-21. On PND-26, subjects were assessed open-field activity (OFA) and conditioned and tested as adolescents or adults using the SE-CPP protocol between PND-28 to -32. The protocol was counterbalanced so each animal received one injection of ethanol and one of saline in either of two distinct contexts on day 1 or day 3 of a five-day conditioned place preference experiment (days 2 & 4 mice were left undisturbed in their home cages) and tested for conditioned bias on day 5 following a saline injection. In adolescent mice, the effect of maternal separation depended on strain and sex. In KO females, maternal separation decreases initial rewarding effects of ethanol; whereas, in B6 females had no effect on initial subjective reward to alcohol. These data suggest that b-endorphin mitigates the effect of maternal separation on alcohol reward during adolescence. These data support the contention that sex differentially impacts vulnerability to effect of stress on alcohol use disorders.

Disclosures: M.R. Scopano: None. **H.E. Jones:** None. **J.E. Grisel:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.03

Topic: F.04. Stress and the Brain

Title: The effects of postnatal maternal ethanol consumption on maternal care and offspring anxiety-related behaviors

Authors: *A. E. HOWARD, C. M. KREITLER, S. L. P. LIPPI;
Angelo State Univ., San Angelo, TX

Abstract: Children of alcoholic parents are at a greater risk of experiencing neglect and/or developing a mental illness later in life (Mosel, 2021). The current research was to assess the effects of ethanol use on maternal care and offspring anxiety-related behaviors using a mouse model. Maternal behavior is a common measurement of neglect in mice. Three C57BL/6J female mice were used to breed in-house and were randomly separated into one of two conditions: control or ethanol administration (EA). This study replicated an intermittent two-bottle choice paradigm, one of water and the second of 10% ethanol solution, to simulate voluntary binge drinking in rodents. The amount of time interacting with one's pups, time spent nest building, and time away from their nest over a 30-minute period was recorded as maternal behavior. After weaning on PND 21, the offspring from each mother were tested using the Open Field Test (OFT), Elevated Zero Maze (EZM), and Forced Swim Test (FST) starting on PND 28 to measure anxiety and depression-like behaviors. An independent sample t-test (ethanol v. control) was run for each of the behavioral tests. A paired-sample t-test was run to compare weights of the dams' drinking bottles to determine how much water and ethanol was drunk. The results found were not in support of the initial hypothesis. The EA mothers drank significantly more water than ethanol throughout the administration period, which may have contributed to the insignificant maternal behavior results and lack of anxiety and depression-like behaviors in the EA offspring. This study showed that limited binge drinking has little effect on offspring anxiety symptoms during adolescence.

Disclosures: A.E. Howard: None. **C.M. Kreitler:** None. **S.L.P. Lippi:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.04

Topic: F.04. Stress and the Brain

Support: NIH RO1-MH115900

Title: Sex differences in resilience induced by chronic, predictable stress in mice

Authors: L. KENNEY[1], *S. SMITH[2];
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Abstract: Most studies investigating stress during adolescence focus on its negative effects, while few explore its benefits during this critical period. Rodent studies mostly utilize males, despite evidence that females have a higher risk of developing mood and anxiety disorders. The goal of this study was to explore the effect of chronic, predictable stress during the pubertal period in both male and female mice on measures of anxiety and depression. Mice were restrained for 2 hours/day over 2 weeks, beginning at the onset of puberty (PND 35 for females and PND 38 for males). They were then tested with the elevated plus maze (EPM) and forced swim test (FST) to assess avoidance behavior and coping strategy, respectively. Restrained female mice were significantly less immobile in the FST compared to controls (R: 37% ± 0.04, C: 56% ± 0.04, $t(26)= 3.6$, $p= 0.001$), suggesting development of resilience to depressive behavior. Interestingly, this effect was not seen in male mice, as restrained and control mice were equally immobile (R: 58% ± 0.12, C: 52.6% ± 0.17, $t(17)= 0.69$, $p=0.50$). Altogether, results indicate that chronic, predictable stress during puberty prevents a depressive phenotype in female mice only.

Disclosures: L. Kenney: None. **S. Smith:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.05

Topic: F.04. Stress and the Brain

Support: R01MH115914
R01MH115049

Title: Early life adversity alters reward learning and decision making mechanisms in mice

Authors: *M. GALLO[1], A. HAMID[3], A. JASKIR[1], T. PAN[1], D. OFRAY[4], M. J. FRANK[5], C. I. MOORE[2], K. G. BATH[6];
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Abstract: Exposure to early life adversity (ELA) is associated with heightened risk for the development of anhedonia, depression and substance use disorder. Adverse outcomes may reflect ELA-linked impacts on reward pursuit including blunted reward sensitivity, more habitual control of behavior, and steeper delayed discounting. However, the effects of ELA on the development of reward-motivated behavior and their neural underpinnings remain poorly understood. Here, in a probabilistic bandit task, we studied the sensitivity of mice raised under

control or ELA conditions to reward contingencies and rates of adaptation to changing contingencies across reward rich and reward poor environments. Overall, mice adapted their choice patterns when reward contingencies changed and were also sensitive to environmental richness as defined by the frequency of rewards in recent history. Importantly, mice exposed to ELA adapt more slowly when contingencies changed and showed deficits in sensitivity to reward contingencies as a function of environmental richness. Ongoing computational modeling and molecular work will explore the link between behavioral outcomes and striatal dopamine function.

Disclosures: M. Gallo: None. **A. Hamid:** None. **A. Jaskir:** None. **T. Pan:** None. **D. Ofray:** None. **M.J. Frank:** None. **C.I. Moore:** None. **K.G. Bath:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.06

Topic: F.04. Stress and the Brain

Support: R01MH115914

Title: Sex-differences in threat anticipation and related circuitry as a consequence of early life adversity

Authors: *C. DEMAESTRI[1], D. OFRAY[1], M. CRITZ[1], K. G. BATH[2];
[2]Psychiatry, [1]Columbia Univ., New York, NY

Abstract: Experiencing early life adversity (ELA), such as resource scarcity, is associated with increased risk for anxiety disorders, with females having a two-fold greater risk compared to males. A core feature of many anxiety disorders is excessive anticipation of threat. In particular, the startle response is a suitable and translational behavior to study threat anticipation because it is reliably enhanced when threat is cued and it is conserved across species. ELA alters the development of threat circuitry, with excessive recruitment of the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) as well as altered expression of corticotropin-releasing hormone (Crh). Here, we sought to test for sex- and ELA-specific changes in activation of Crh cells within these regions after mice engage in a task that tests threat responding. The goal is to better understand the neural mechanisms of excessive threat anticipation. We used the limited bedding model of ELA to test its effects on startle response. We found that mice startled to a loud noise when that noise was cued by a conditioned tone, suggesting accurate elevation of vigilance. However, female ELA mice also showed enhanced startle when threat was uncued, suggesting heightened reactivity. We carried out studies assessing immediate early gene activation, Crh cell number and the overlap of these two markers in the BNST and CeA to test

for possible mechanistic underpinnings of this sex disparity in startle response. This work will lay the groundwork for future studies aimed at identifying sex-specific vulnerability to pathology and will contribute to the development of effective treatments that are individualized to the experience and sex of the individual.

Disclosures: C. Demaestri: None. **D. Ofray:** None. **M. Critz:** None. **K.G. Bath:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.07

Topic: F.04. Stress and the Brain

Support: Deutsche Forschungs Gemeinschaft (DFG)

Title: Fkbp51 in glutamatergic forebrain neurons mediates beneficial effects of moderate early life adversity on hippocampal structure and function.

Authors: L. VAN DOESELAAR, H. YANG, S. MITRA, J. BORDES, C. ENGELHARDT, B. M. LEA, J. DEUSSING, B. BOULET, M. CZISCH, M. EDER, M. V. SCHMIDT;
Max Planck Inst. of Psychiatry, Muenchen, Germany

Abstract: Exposure to stressors in early in life can be a risk factor for later psychopathologies, but may also enhance stress resilience, dependent on the genetic background and later life conditions of an individual. The chaperone FKBP51 has been implicated in mediating such effects, but the underlying mechanism are still largely unclear. Furthermore, recent research has highlighted the differential responses to stress between sexes. In this study, we addressed the question whether early-life stress exposure can enhance cognitive performance in adulthood and if FKBP51 plays a role in this process. Mice with a conditional deletion of FKBP51 in forebrain glutamatergic neurons ($FKBP51^{Nex}$) or wild-type (WT) litter mates were either subjected to early life stress in the form of limited nesting and bedding material (ELS) or left undisturbed. Cognitive testing in adulthood at 5 - 7 months of age revealed that predominantly in female offspring ELS resulted in an improved cognitive performance specifically under high-stress conditions, and this effect was absent in $FKBP51^{Nex}$ mice. Structural MRI following the behavioral tests revealed genotype or ELS-induced volumetric changes in cortical brain regions or the BNST and cerebellum, respectively. In addition, RNA bulk sequencing revealed interesting differences in expression profiles within the ventral hippocampus of female mice, whereas field potential recordings in CA1 dorsal hippocampal slices of a separate cohort of female $FKBP51^{Nex}$ and WT mice exposed contrasting LTP induction profiles in response to a corticosterone application. Taken together, our results show that moderate exposure to early life adversity can enhance resilience in a sex-dependent manner by improving cognitive performance

under high stress conditions as a result of molecular, functional and structural changes. By further unraveling the structural and molecular pathways responsible for this pro-resilient phenotype, we will gain valuable insights in potential intervention and treatment strategies.

Disclosures: L. van Doeselaar: None. **H. Yang:** None. **S. Mitra:** None. **J. Bordes:** None. **C. Engelhardt:** None. **B.M. Lea:** None. **J. Deussing:** None. **B. Boulet:** None. **M. Czisch:** None. **M. Eder:** None. **M.V. Schmidt:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.08

Topic: F.04. Stress and the Brain

Support: KIDS2HEALTH

Title: Inhibition of the psychiatric risk factor FKBP51 reverses long-term effects of early life stress on social behavior

Authors: *J. BORDES[1], S. KARAMIHALEV[1], L. MIRANDA[1], M. PÖHLMANN[1], L. BRIX[1], L. VAN DOESELAAR[1], C. ENGELHARDT[1], J. DEUSSING[1], G. WINTER[2], F. HAUSCH[3], B. MÜLLER-MYHSOK[1], A. CHEN[1], M. V. SCHMIDT[1];
[1]Max Planck Inst. of Psychiatry, Munich, Germany; [2]Ludwig-Maximillians-Universität, Munich, Germany; [3]TU of Darmstadt, Darmstadt, Germany

Abstract: Stress-related disorders are drastically increasing the burden on society. In particular depression has been described by the WHO as the leading cause of worldwide disability and affects an estimated 264 million people. However, the classical anti-depressant treatment options rely on a poorly understood mechanism which show an enormous variability in efficacy and high numbers of relapse. Interestingly, human genetic studies observe a profound link between the FKBP5 gene (coding for the FKBP51 protein) and the risk for stress-related disorders. The current study investigates the role of the novel anti-depressant drug target, the FKBP51 inhibitor SAFit2 as a potential novel drug-treatment for stress-related disorders. Mice were exposed to early life stress (ELS) using limited nesting and bedding material and were treated with SAFit2 during the entirety of the stress protocol. Male offspring of stress or control litters with or without treatment were then tested for social behavior during adolescence and adulthood in the social box, an enriched environment allowing continuous tracking of multiple mice over several days. Exposure to ELS significantly affected the hierarchical position of the animals during adolescence and adulthood, with stressed animals being more likely to become subordinate compared to nonstressed mice. Interestingly, this effect was reversed by the treatment with SAFit2 in stressed mice. Moreover, a similar effect was observed on social approaches, in which

stressed animals showed a lower social interest then nonstressed and stressed, SAFit2 treated animals. In conclusion, the current study shows that ELS has a strong effect on social behavior in mice, leading to an alteration in social hierarchy. This effect can be fully rescued by administering the FKBP51 antagonist SAFit2 during the stress exposure. Future experiments include to investigate short-term social behavioral effects using DeepLabCut analysis in a social interaction task and investigating the molecular profile using RNA sequencing in important brain regions for social behavior and stress. The current study underlines the importance of FKBP51 in stress physiology and stress-related disorders. Importantly, the results further encourage the clinical development of FKBP51 inhibitors for the treatment of stress-related disorders.

Disclosures: J. Bordes: None. **S. Karamihalev:** None. **L. Miranda:** None. **M. Pöhlmann:** None. **L. Brix:** None. **L. van Doeselaar:** None. **C. Engelhardt:** None. **J. Deussing:** None. **G. Winter:** None. **F. Hausch:** None. **B. Müller-Myhsok:** None. **A. Chen:** None. **M.V. Schmidt:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.09

Topic: F.04. Stress and the Brain

Title: Evaluation of the anxiolytic effect of gardenin A

Authors: *M. J. RODRÍGUEZ LÓPEZ[1], L. V. CÓRTES MENDOZA[1], Á. J. ALONSO CASTRO[1], D. GASCA MARTÍNEZ[2], C. ALBA BETANCOURT[1], M. A. DEVEZE ÁLVAREZ[1];
[1]Univ. de Guanajuato, Guanajuato, Mexico; [2]Unidad de Análisis Conductual, Univ. Nacional Autónoma de México, Instituto de Neurobiología, Mexico

Abstract: Objective To determine the anxiolytic effect of the compound Gardenin A using unconditioned anxiety tests. **Justification** The drugs used for the treatment of anxiety disorder have severe adverse effects, which has led to the search for new anxiolytic substances. Medicinal plants have presented great potential as a source of new bioactive substances. Gardenin A (GA), extracted from the *Gardenia gummifera* plant, exerts antihyperlipidemic and hepatoprotective effects against non-alcoholic fatty liver disease, in addition to being a vessel-relaxing and antioxidant. GA is an important component of medicinal plants with anxiolytic and antidepressant effects, in the present investigation, this function was evaluated with unconditioned behavioral tests. **Methodology** We used three groups of eight mice each, from the CD1 strain. The mice were administered 100 µL of an oral GA compound, using concentrations of 1, 10, and 25 mg/kg per weight. After one hour of administration of the compound, the mice were subjected to three unconditioned tests (elevated plus maze, light/dark box, open field) to

generate anxiety and so evaluate if the compound decreases it. The results are compared with positive control of clonazepam and saline solution (vehicle). **Results** In the elevated plus maze, mice treated with the three different concentrations of GA showed similar behavior to mice treated with clonazepam, by showing an increase in the number of entries, exploration time, and distance traveled in the open arms. No significant differences were observed concerning the three concentrations tested. In addition, the dwell time and the distance traveled in the closed arms was less, compared to the vehicle. In the light/dark box the mice treated with GA showed a greater number of entries in the illuminated area of the camera, as well as they stayed longer in it. In the open field, mice treated with GA and with clonazepam stayed longer and traveled a greater distance in the central area, compared to the vehicle that stayed longer in the periphery area (safe zone). **Conclusions** The results suggest that GA induces anxiolytic effects in mice subjected to unconditioned anxiety tests, as behaviors similar to clonazepam (anxiolytic agent) were observed.

Disclosures: M.J. Rodríguez López: None. **L.V. Córtes Mendoza:** None. **Á.J. Alonso Castro:** None. **D. Gasca Martínez:** None. **C. Alba Betancourt:** None. **M.A. Deveze Álvarez:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.10

Topic: F.04. Stress and the Brain

Support: NSF CREST PRCEN #173619
NIH/NIMHD #S21MD001830
NIH/NIAAA- #1R01AA027808-01A1

Title: Effects of bisphenol-a (BPA) exposure on anxiety behaviors necessary for survival

Authors: *K. CARDONA[1,2], C. VELÁZQUEZ[2], K. VEGUILLA[2], A. RIVERA[2];
[1]Anat. and Neurobio., Univ. of Puerto Rico- Med. Sci. Campus, San Juan, PR; [2]Inst. of Neurobio., San Juan, PR

Abstract: Bisphenol-A (BPA) is an anthropogenic stressor mass-produced to assist in the manufacturing of almost all plastics and resins since the 1960s. Plastic pollution has become an increasing concern over the past six decades with BPA production surpassing 7 billion kilograms per year. It is common to encounter BPA in air, dust, food, and water. Heat and changes in pH cause this chemical to leach out of products and spread throughout the environment. Important environmental organizations like EPA, have declared BPA as the third-highest priority environmental hazard. The concentrations of BPA in the environment vary depending on location, its temperature, pH, source, and time of sampling. For example, BPA has been detected

in effluents in the United States at concentrations of 12 µg/L. The current U.S. EPA (Environmental Protection Agency) reference for Lowest Observed Adverse Effect Level (LOAEL) is 50 mg/kg/d, of BPA. Previous studies have shown that at low BPA concentrations, i.e. 2 mg/kg/day, there are some effects on the development of secondary sexual characteristics and neurotoxic effects in the brain and behavior. Specifically, neurologic effects have been noted after early-life exposure to BPA in the rodent brain, producing deficits in social behavior, cognitive impairment, and increases in anxiety and depression-like behavior. All of these deficits compromise the innate ability to face challenges and limits chances for survival. Furthermore, current research has determined a link between BPA-induced epigenetic changes in early childhood, leading to increased DNA methylation of Fkbp5 gene and stress-induced expression, associated to increased sensitivity to stress and the risk of developing PTSD and anxiety behaviors. Despite these potential indicators, there has been no research directly addressing the development of anxiety-like behavior in males in response to BPA exposure at early stages of development. Our preliminary data with adult male mice show a decrease in fear learning when exposed to Bisphenol-A (50ppm). Therefore, ***our hypothesis states that early exposure to BPA increases the likelihood of developing anomalies in stress response correlated to the development of anxiety-like disorders***. Our results showed that expression of fear in adolescent mice is significantly more in Bisphenol-A exposed mice (50ppm) than the control group during extinction learning. These results provide novel evidence for the capacity of common contaminants such as BPA, at physiologically relevant concentrations, to lower the threshold for neurological disorders in response to challenges, specifically learning in adolescent mice.

Disclosures: K. Cardona: None. **C. Velázquez:** None. **K. Veguilla:** None. **A. Rivera:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.11

Topic: F.04. Stress and the Brain

Title: Evaluation of the anxiolytic effect of a pseudobulb extract of Stanhopea tigrin

Authors: *M. GONZÁLEZ QUEVEDO[1], A. J. ALONSO CASTRO[1], C. ALBA BETANCOURT[2], M. A. DEVEZE ÁLVAREZ[3], D. GASCA MARTÍNEZ[4];
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Abstract: Objective To determine the anxiolytic effect of Stanhopea tigrina pseudobulb extract using unconditioned anxiety tests.
JustificationAnxiety is a health problem that can become disabling and has increased because of COVID-lock down. The available treatments have adverse side effects, so the search for new

options, particularly from natural sources, becomes necessary. Orchids are used to cure dysentery, cough, insolation, headache, fever and has been attributedanti-inflammatory properties. *Stanhopea tigrina* is an orchid endemic to Mexico. Its flowers were used to treat insolation and weakness. Pseudobulbs are the storage area and source of nutrients of orchids, as they conserve water, carbohydrates and micronutrients, being a fundamental part of these plants. In this work we propose to evaluate the possible anxiolytic properties of this plant structure as an approach to propose an alternative treatment for anxiety.
MethodologyThree concentrations of a pseudobub extract of *Stanhopea tigrina* (10, 50 and 100 mM)were tested in 3 groups of 10 CD1 mice, the extract was orally administered. The results were compared with clonazepam (anxiolytic drug, positive control) and saline solution (vehicle).After one hour of administration of the compound, the mice were placed in 3 different unconditioned tests (elevated plus maze, light/dark box, open field) to evaluate the possible anxiolytic effect of the orchid.
ResultsIn the plus maze, the mice treated with the extract stayed longer and entered many more time the area of the open arms, compared to the vehicle. Similarly, they also explored the closed arms of the labyrinth, without showing significant differences with mice treated with clonazepam. In the light/dark box, the mice treated with the extract showed a number of entries to the illuminated area similar to clonazepam and it seemed dose-dependent.
In the open field the mice traveled a total distance and to the center area similar to clonazepam, being also dose-dependent.
ConclusionsThe results suggest that the pseudobulb extract of *S. tigrina* induces anxiolytic effects in mice evaluated in unconditioned tests.

Disclosures: M. González Quevedo: None. **A.J. Alonso Castro:** None. **C. Alba Betancourt:** None. **M.A. Deveze Álvarez:** None. **D. Gasca Martínez:** None.

Poster

P597. Early-Life Stress: Effects on Anxiety, Social Function, Reward, and Depression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P597.12

Topic: F.04. Stress and the Brain

Support: SIP-20210049

Title: Anxiolytic-sedative activity of Sargassum fluitans and Sargassum buxifolium: an experimental study

Authors: *S. A. GIL-LÓPEZ[1], M. DÉCIGA-CAMPOS[1], E. HERNÁNDEZ-NUÑEZ[2];
[1]SEPI, Escuela Superior de Medicina, Inst. Politécnico Nacional, Mexico City, Mexico; [2]Dept. de Recursos del Mar, Cinvestav Mérida, Mérida, Mexico

Abstract: Sargasso is constituted by a mixture of macroalgae that are found in the Ocean. Currently, in Mexico, *Sargassum* has been concentrated on the coasts of Yucatán and Quintana Roo. The influx of *Sargassum spp.* into the Mexican Caribbean Sea representing health, economic and environmental problems. This study aimed to characterize the anxiolytic, anticonvulsant, and sedative activity of *Sargassum fluitans* and *Sargassum buxifolium* MeOH extracts. Mice administered with various doses of *Sargassum fluitans* (30-600 mg/kg i.p.), *Sargassum buxifolium* (30-600 mg/kg i.p.), or diazepam (2 mg/kg, i.p.; a positive control) were assessed in ambulatory activity (cylinder of exploration, open-field, and hole-board tests), potentiation of the sodium pentobarbital-induced hypnosis and seizures-induced pentylenetretrazol (PTZ) assays. Results showed that both MeOH extracts gradually decrease the number of explored squares, surveys and perforations explored. Thus, *Sargassum fluitans* and *Sargassum buxifolium* showed a dose-dependent anxiolytic-like effect. In contrast, both species of Sargassum neither modify the sedative-hypnotic activity nor reduce the seizures-induced PTZ. Mass-spectrometry analysis showed that *Sargassum fluitans* extract has 64 compounds and *Sargassum buxifolium* extract 71 compounds. Some of these compounds are related to anxiolytic effects (-(+)-Ascorbic acid 2,6-dihexadecanoate; Phytol; Octadecanoic acid; ς-Tocopherol; Ergostanol; Stigmasterol;dl-à-Tocopherol). The results support further investigation of *Sargassum* as a potential anxiolytic phytomedicinal agent. In addition, it offers the opportunity to continue studying other pharmacological activities of the *Sargassum* related to the central nervous system and their therapeutic use.

Disclosures: S.A. Gil-López: None. **M. Déciga-Campos:** None. **E. Hernández-Nuñez:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: CHR

Title: Altered functional connectivity during acute global hypoxia using calcium imaging in mice

Authors: *M. BAKKER[1], I. DJEROUROU[2], S. BELANGER[4], F. LESAGE[5], M. P. VANNI[3]; [1]Biomed. Engin., Polytechnique de Montréal, Montréal, QC, Canada; [2]École d'Optométrie, Univ. de Montréal, Montréal, QC, Canada; [3]Univ. de Montréal, Montreal, QC, Canada; [4]Labeo Technologies, Montreal, QC, Canada; [5]Ecole Polytechnique Montreal, Montreal, QC, Canada

Abstract: A lack of oxygen in tissue, or hypoxia, can occur as a result of sleep apnoea, COVID-19 implications, and stroke, among others. Hypoxia is related to a lower cognition, both in

chronic and acute situations. How this decrease in cognition comes into play is still unknown, but might be related to altered functional connectivity of the brain. Resting state connectivity assesses the way the brain is communicating with itself in the absence of stimuli and has been shown to be altered in some diseases, such as schizophrenia, multiple sclerosis or Alzheimer's. Among changes linked to cognition, the amount of connectivity between hemispheres has been frequently used as a biomarker. However, previous studies show contradicting results; some find cognition correlated with increased interhemispheric connectivity while others find the opposite. Most of these studies were done with fMRI, which measures BOLD response and not direct neuronal activity. This may explain the contradictory results, since BOLD measurements in diseases that alter vasculature in the brain might not be a good proxy for neuronal activity. Here, imaging techniques directly measuring the neuronal activity, such as calcium imaging, may help shed light on these questions. In this study, we used calcium and intrinsic optical imaging in mice to investigate changes in connectivity during acute global hypoxia. First, mice were injected (IV) with a virus allowing the expression of GCaMP6 in the dorsal cortex and were implanted with a chronic window. After a recovery period, we imaged the fluctuations of fluorescence and reflectance signals in the cortex during resting state in different settings: normoxia (21%), light hypoxia (12%), mild hypoxia (10%) and severe hypoxia (8%). The fluorescence intensity was corrected from hemodynamic signals and the standard deviation was calculated for each pixel as a proxy of neuronal activity. Then, correlation values between the centroids of cortical functional areas were calculated and used to assess the connectivity of inter- and intrahemispheric networks. During hypoxia, we observed a general decrease in neuronal activity. This decrease was spatially heterogeneous. Furthermore, we found altered interhemispheric and intrahemispheric connectivity. Since this change in connectivity is not measured with hemodynamic proxies, these preliminary results give a more robust substantiation of the idea that interhemispheric connectivity is related to cognitive functioning. Additionally, we shed light on the functional connectivity changes that occur during hypoxia, thereby providing important fundamental knowledge for COVID-19, Alzheimer's, and aging, among others.

Disclosures: M. Bakker: None. **I. Djerourou:** None. **S. Belanger:** None. **F. Lesage:** None. **M.P. Vanni:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01 AG047972-01A1

Title: Relationships between cerebral metabolic rate of oxygen consumption during task and at rest in older adults

Authors: *D. ABDELKARIM[1], Y. ZHAO[1], M. P. TURNER[1], K. WEST[1], D. SIVAKOLUNDU[2], P. LIU[3], H. LU[3], B. P. RYPMA[1];
[1]Behavioral & Brain Sci., The Univ. of Texas at Dallas, Dallas, TX; [2]Yale Sch. of Med., New Haven, CT; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Brain aging processes are complex, involving many interacting physiological systems. Age-related changes in metabolic processes arise from altered pathways and cellular damage that increase steadily over the lifespan. It is known that the resting cerebral metabolic rate of oxygen consumption ($CMRO_2$) increases with age while $CMRO_2$ during task decreases. In this study, we aimed to compare these divergent processes and their relationships to other processes of brain aging. In groups of younger (20-35) and older (55-70) adults, we used the T2-relaxation under spin tagging (TRUST) technique along with arterial phase contrast-derived cerebral blood flow to estimate resting $CMRO_2$, and we calculated $CMRO_2$ during a simple visual task using a dual-echo sequence and hypercapnia gas challenge. During the visual task, participants viewed a checkerboard flickering at a rate of 2Hz, 4Hz or 8Hz. Volumetric measurements were calculated based on high-resolution anatomical scans. We found that resting $CMRO_2$ differs between age groups. In older adults, there were significant anticorrelations between resting $CMRO_2$ and task-based $CMRO_2$ across flicker frequencies. No such relationships were observed in younger adults. These age-differences were further explored using other physiological data such as atrophy estimates, blood flow, and neural-vascular coupling. These results suggest that aging exerts metabolic demands upon neural processes at rest and contribute to the resources demanded during task performance. Such resource demands would be expected to compromise those hyperemic systems involved in guiding metabolic resources to active parenchyma in older adults.

Disclosures: D. Abdelkarim: None. **Y. Zhao:** None. **M.P. Turner:** None. **K. West:** None. **D. Sivakolundu:** None. **P. Liu:** None. **H. Lu:** None. **B.P. Rypma:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant T32-GM008764
Simons Collaboration on Plasticity in the Aging Brain
NIH Grant R01-AG070135

Title: Resting state fMRI signals contain spectral signatures of local hemodynamic response timing

Authors: *S. M. BAILES, L. D. LEWIS;
Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: While neural activity fluctuates on timescales of milliseconds, the subsequent changes in blood oxygenation measured by fMRI occur over several seconds, referred to as the hemodynamic response function (HRF). Both the shape and timing of the HRF vary substantially across the brain, and proper characterization of these variations is critical for the interpretation of the blood oxygenation level dependent (BOLD) signal obtained from fMRI. Despite this, most current studies do not map local HRF timing due to the limitations of current techniques for estimating vascular delays. We aim to test if the information from the spectrum of resting state fMRI can be used to map vascular delays across the brain. Resting state scans are acquired in most studies and require no task performance from the subject; thus, using information from the resting state signals would provide a simple and effective way of understanding the temporal dynamics of the HRF across the brain. To test whether the spectrum of resting state fMRI signals should contain signatures of HRF timing, we first used HRFs with varying timings to generate simulated BOLD responses. Analysis of the spectra of these simulated BOLD responses showed voxels with faster dynamics exhibited less power in low frequency bands but a shallow decline at higher frequencies with the difference being most prominent <0.2 Hz. Next, we verified this finding in fast fMRI data collected at 7 Tesla. We quantified the phase delay of voxels in the primary visual cortex in response to an oscillating stimulus and identified groups of fast and slow voxels. We found significant differences in features derived from the resting state spectra between fast and slow voxels, quantified by the slope of the spectrum under 0.2 Hz, the mean power under 0.1 Hz, and the exponent of the aperiodic 1/f fit. Lastly, to investigate brain-wide differences in spectral features in a large dataset, we examined a subset of the Human Connectome Project Aging dataset, and calculated the slope of the resting state spectra <0.2 Hz across the brain. We found that deeper brain structures consistently had more shallow slopes compared to cortical structures across all subjects. Taken together, our results demonstrate that temporal properties of the HRF affect the spectral features of spontaneous fMRI signals. We find that this insight enables us to distinguish voxels and regions exhibiting faster or slower hemodynamic responses using only resting state information. This can allow researchers to better understand and predict the temporal properties of the HRF within individuals and large groups on both voxel-wise and regional levels, which is crucial for accurate fMRI analyses.

Disclosures: S.M. Bailes: None. **L.D. Lewis:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.04

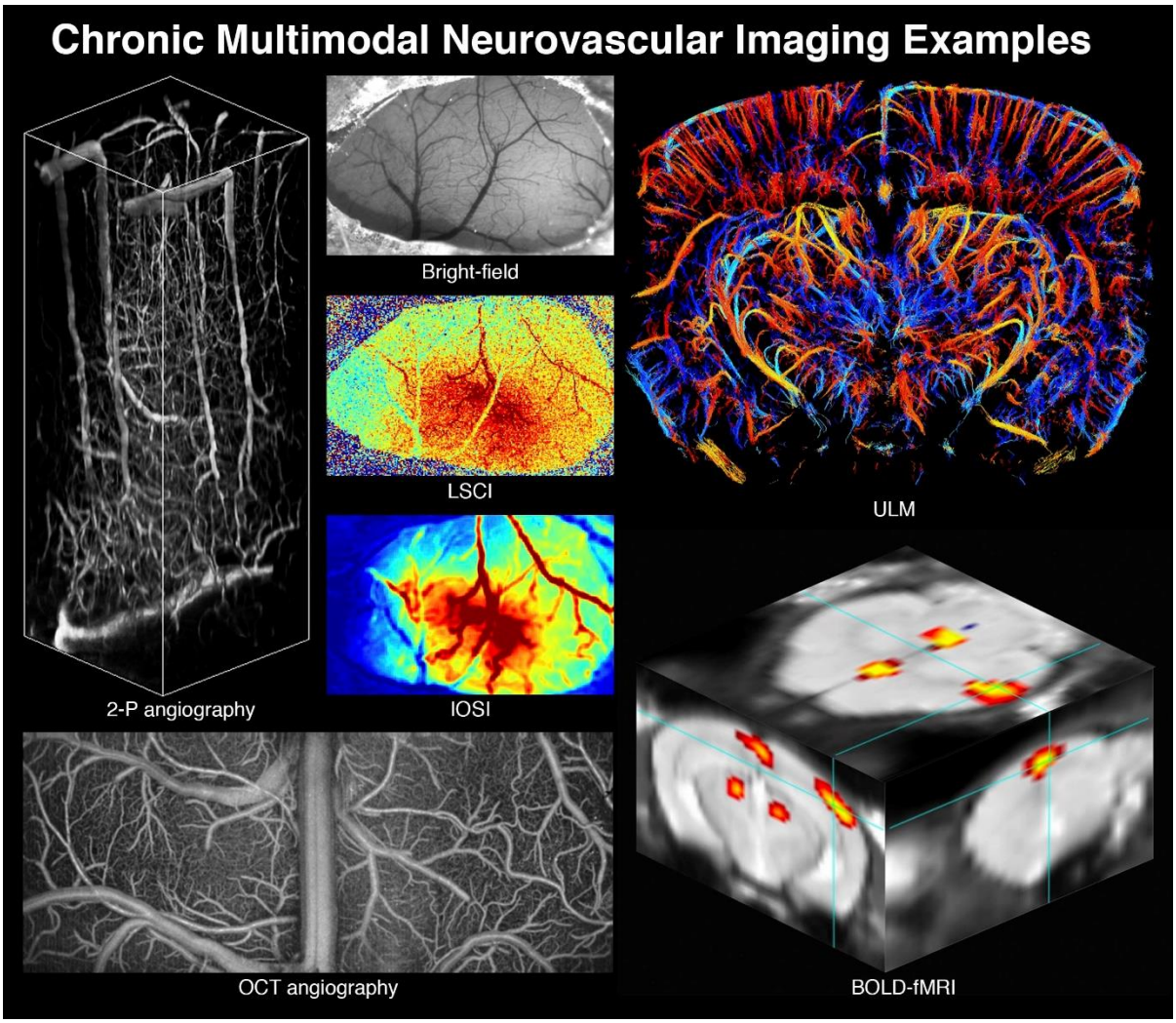
Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01MH111359
NIH Grant R01NS108472
NIH Grant R01NS057198
NIH Grant K99AG063762

Title: Chronic Cranial Windows for Long Term Multimodal Neurovascular Imaging in Mice

Authors: K. KILIÇ[1], M. DESJARDINS[2], J. TANG[3], M. THUNEMANN[1], S. SUNIL[1], E. ERDENER[4], D. D. POSTNOV[5], P. DORAN[1], D. A. BOAS[1], A. DEVOR[1];
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Abstract: The utilization of multiple imaging modalities in experimental neurosciences enables comprehensive investigation of brain structure and function in mice. Each imaging technology has unique advantages and disadvantages: for example, while ultrasound allows whole-brain imaging of cerebral blood flow, it cannot achieve micrometer resolution. On the other hand, two-photon microscopy yields micrometer resolution but is limited by relatively shallow penetration (in common practice, limited to the upper cortical layers in brain) and a relatively small field of view (usually, <1 mm). Combination of multiple imaging modalities offers measurements of a wide spectrum of neuronal, glial, vascular, and metabolic parameters needed for comprehensive investigation of physiological and pathophysiological mechanisms. Previous studies have described a variety of designs and surgical implantation techniques for chronic cranial windows in mice enabling longitudinal measurements. These window implants allow imaging of awake mice, which is important for investigation of brain function without the confounding effects of anesthesia on the physiology of nervous and cardiovascular systems. Although some of these protocols are very detailed, they usually employ imaging with one modality. With this study we expanded on these previous publications and describe a spectrum of surgical methods in mice suited for multiple imaging modalities, used alone or in combination, including two-photon microscopy, laser speckle contrast imaging, intrinsic optical signal imaging, optical coherence tomography, and functional US. Our optical windows can last for a span of up to 6 months after surgery and can be made MRI-safe for combined optical imaging or optogenetic (OG) stimulation in awake mice undergoing fMRI. We applied the presented procedures to investigate physiological mechanisms as well as animal models of disease (such as stroke). Following these surgical techniques and practices will yield higher experimental success and reproducibility of results and decrease the number of animals needed.



Disclosures: K. Kiliç: None. **M. Desjardins:** None. **J. Tang:** None. **M. Thunemann:** None. **S. Sunil:** None. **E. Erdener:** None. **D.D. Postnov:** None. **P. Doran:** None. **D.A. Boas:** None. **A. Devor:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.05

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIMH Grant 1K01MH096175-01
National Institute of Diabetes and Digestive and Kidney Diseases
Oklahoma Tobacco Research Center grant

Title: Dietary fat, but not obesity, significantly affects brain reward activity to visual food cues

Authors: *I. GALLAGHER[1], V. L. DARCEY[1], J. A. AVERY[3], J. GUO[1], A. B. COURVILLE[2], W. K. SIMMONS[4], J. E. INGEHOLM[3], A. MARTIN[3], K. D. HALL[1];
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Abstract: The neural mechanisms underlying the biological, behavioral, and environmental influences on the development of obesity remain unclear. Functional neuroimaging studies have led to inconsistent results, with some studies suggesting that people with obesity have greater response to visual food cues in brain regions associated with reward processing, whereas others report no significant group differences. The aim of this study was to determine whether people with obesity, compared to those without obesity, have altered brain activity in reward regions in response to visual food cues during an fMRI pleasantness task in a highly controlled, inpatient setting. On the 4th day of a standardized weight maintenance diet (50% of calories from carbohydrate, 35% fat, 15% protein), participants completed fMRI scans, 3 hours after their last meal. The scan included an event-related food pleasantness design with 144 food images, varying from highly processed, high calorie foods to minimally processed/raw low calorie produce. Participants were asked "If given the opportunity right now, how pleasant would it be to eat this food?" and responded on a scale from 1 to 7, with 1 "*neutral*" and 7 "*extremely pleasant*". Neuroimaging preprocessing and group-level analyses (3dttest++) were performed using Analysis of Functional Neuroimaging tools. Predictive BOLD signals were amplitude modulated by participant's pleasantness ratings for each food image. Analyses were conducted using a reward circuit mask for small volume correction. The study enrolled 20 adults with obesity (35.3±7.5 years old; BMI 36.6±4.6 kg/m^2) and 22 adults without obesity (27.7±6.2 years old; BMI 22.5±2.8 kg/m^2). During the fMRI task, though people with obesity rated all foods more pleasant than participants without obesity (4.5±0.5 vs 4.0±-0.8, p=0.016), there were no group differences in brain activity to visual food cues . Given absence of group differences, we found that, among the full, combined sample (n=42), brain regions responsive to subjective pleasantness of visual food cues were similar to our previously published work examining solely the control group without obesity. Exploratory regression analyses using 3-day food records of participants free-living diets suggest that greater percent of calories from fat in participants usual diet is associated with greater left ventral striatal response to highly pleasant visual food cues ($p<0.0001$, $R^2 = 0.25$). In summary, dietary fat may modulate reward-related brain activity to visual food cues, but contrary to our hypothesis, there were no significant differences in brain activity to visual food cues between people with and without obesity.

Disclosures: I. Gallagher: None. **V.L. Darcey:** None. **J.A. Avery:** None. **J. Guo:** None. **A.B. Courville:** None. **W.K. Simmons:** None. **J.E. Ingeholm:** None. **A. Martin:** None. **K.D. Hall:** None.

Poster

P623. Functional Imaging: II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P623.06

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R00-MH111748
NIH R01-AG070135
Searle Scholars Award
NSF BCS-1625552

Title: Neural activity drives macroscopic cerebrospinal fluid flow in the awake human brain

Authors: *S. D. WILLIAMS, N. E. FULTZ, N. TACUGUE, Z. VALDIVIEZO, B. SETZER, L. D. LEWIS;
Boston Univ., Boston, MA

Abstract: The circulation of cerebrospinal fluid (CSF) in the brain facilitates the exchange of waste products with fresh nutrients to maintain brain homeostasis. Given the importance of CSF flow in the clearance of potentially toxic macromolecules, identifying the underlying mechanisms that drive fluid flow could enable modulation of these critical fluid dynamics that support brain health. Several drivers of CSF flow have been identified, including systemic respiratory and cardiac-linked mechanisms. In addition to these mechanisms, recent work in humans has also demonstrated there may be a neural driver of macroscopic CSF flow. Waves of neural activity precede macroscopic CSF flow through the fourth ventricle during non-rapid eye movement sleep. This coupling suggests that in addition to the well-characterized systemic physiological drivers of flow, macroscopic CSF flow may also be driven by neural mechanisms. To test whether the temporal coupling between neural activity and CSF flow reflects a causal relationship, we used sensory stimulation to drive neural activity in the awake human brain and measured corresponding evoked CSF flow. We performed three experiments to test whether neural activity could drive CSF flow, using high-contrast flickering checkerboard visual stimuli to induce widespread neural activity and BOLD responses. First, we collected simultaneous electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) in humans to track the temporal relationship between neural, hemodynamic, and CSF signals. We identified a clear sequence of events: stimulus-locked neural activity recruited waves of global BOLD changes, which alternated with waves of CSF inflow. Next, we replicated our finding that inducing BOLD changes with visual stimuli can evoke CSF flow in a second larger fMRI dataset. Finally, we manipulated the stimuli parameters in a third experiment to test whether stimulus parameters could modulate the amplitude of evoked global cortical and CSF signals. We found that the flicker frequency of the visual stimuli did not significantly alter the amplitude of either the global cortical signal or CSF flow. These results demonstrated that visual stimulation can induce CSF flow, indicating that CSF flow can be driven by neural activity in the

awake human brain. Our findings are consistent with our hypothesis that neural activity drives increases in cerebral blood volume that alternate with compensatory changes in CSF flow. Considering the importance of CSF flow in clearing metabolic waste, future work could investigate whether this neurally-driven flow is associated with significant metabolite clearance in the human brain.

Disclosures: S.D. Williams: None. **N.E. Fultz:** None. **N. Tacugue:** None. **Z. Valdiviezo:** None. **B. Setzer:** None. **L.D. Lewis:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.01

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251

Title: Standardized mapping of sampled sites in the brain involved in energy balance and autonomic control: Developing an a posteriori approach to estimate microdissection accuracy of the rostral ventrolateral medulla

Authors: *S. BALIVADA, G. P. TAPIA, M. J. KENNEY, A. M. KHAN;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: Molecular analysis *in situ* is a valuable tool to identify molecular signatures associated with different brain subregions. For this purpose, a region of interest (ROI) is usually identified *a priori* in unstained or partially stained brain sections using reference landmarks. Although this *a priori* approach assists in sampling the ROI, it does not estimate the sampling accuracy. Here, we proposed an *a posteriori* approach to evaluate the sampling accuracy by mapping the sampled sites within an open-access rat brain reference atlas (*Brain Maps 4.0 (BM4.0); Swanson LW, 2018. J. Comp. Neurol. 526, 935*). To test this approach, the rostral ventrolateral medulla (RVLM) (which corresponds primarily to the *paragigantocellular reticular nucleus lateral part (>1840)* in *BM4.0*) was micropunched from 200 µm-thick fresh frozen hindbrain cryosections collected from male young (3 months), middle-aged (12 months), and aged (24 months) F344 rats (*a priori* approach). For the *a priori* approach, Paxinos and Watson's stereotaxic rat brain reference atlas was used to identify the RVLM-containing sections. The ventrolateral curved edge of the sections and spinal trigeminal tract (Sp5) were used as landmarks. After micropunching, sections were stained with a modified cresyl violet staining procedure. Based on the stained sections' cytoarchitecture, associated *BM4.0* atlas levels were identified, and sampled sites were mapped onto atlas level templates (*a posteriori* approach). Although most of the punches landed in the atlas levels containing the RVLM, we observed a systematic sampling

error in all three age groups. Specifically, more rostral sampling was observed in young and middle-aged rat brain sections (BM4.0 atlas levels 56-65), and more caudal sampling was observed in aged rat brain sections (levels 58-67). These results suggest that standardized mapping of the sampled site as an *a posteriori* approach can be used to evaluate the spatial accuracy of ROI dissection. We plan to use these mapped atlas level templates to develop a quantitative estimate for the ROI dissection accuracy and replicate this *a posteriori* approach using laser capture-microdissected brain sections.

Disclosures: S. Balivada: None. **G.P. Tapia:** None. **M.J. Kenney:** None. **A.M. Khan:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.02

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251
Eloise and Patrick Wieland Graduate Fellowship (KN)
NIH GM069621

Title: Standardized mapping and a high-spatial resolution comparison of the inputs and outputs of the anterior and dorsal regions of the lateral hypothalamic area in the adult male rat using the atlas framework of Brain Maps 4.0

Authors: *A. R. TOCCOLI, D. SOTELO, V. I. NAVARRO, K. NEGISHI, A. M. KHAN; Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: The lateral hypothalamic area (LHA) was long considered to be one large monolithic structure spanning that vast majority of the hypothalamus. However, Swanson (2004; *Brain Maps 3.0*) proposed further provisional subdivisions of this larger structure which were initially based on careful reconsideration of cytoarchitecture. These provisional subdivisions, which are retained in Swanson (2018; *Brain Maps (BM) 4.0*), have since been further tested with the targeted use of tract-tracers to explore if connectional differences between subregions may offer support. These experiments, however, have thus far only covered select subdivisions of the LHA, leaving others untested. We explore the neuroanatomical inputs and outputs of the dorsal (LHAd) and anterior (LHAa) regions of the lateral hypothalamic area (LHA) from *BM4.0* atlas levels 5-41 of the male rat to analyze where these regions differ and overlap in connections. These neuroanatomical connections were made visible with the use of retrograde and anterograde tracers cholera toxin B subunit (CTB) and *Phaseolus vulgaris*-leucoagglutinin (PHA-L) respectively. Inputs and outputs were represented with high-spatial resolution within the atlas templates of *BM4.0* to bring these data into spatial registry with those that have

previously been generated of other LHA subregions which include the juxtaparaventricular (LHAjp), juxtadorsomedial (LHAjd) and suprafornical (LHAs) regions. Thus far these preliminary results seem to demonstrate some variations and overlap in the connectional profiles of the LHAd and LHAa. Although transport of both PHAL and CTB can be seen in various overlapping regions such as the PVT, SUMl, VTA and LH the distributions of these connections within those regions create patterns that are distinct from each other. These regions also show distinctions from previously mapped LHAjp, LHAjd and LHAs subregions. The preliminary results we have observed offer some additional support to the hypothesis that the provisional cytoarchitectural subdivisions of the LHA are connectionally distinct.

Disclosures: A.R. Toccoli: None. **D. Sotelo:** None. **V.I. Navarro:** None. **K. Negishi:** None. **A.M. Khan:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.03

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251

Title: Standardized mapping and high-spatial resolution analysis of dorsal and ventral agranular insular area connections with the endbrain (forebrain) in a reference atlas of the rat brain

Authors: *L. P. MONTES, K. NEGISHI, M. QUINTANA, M. GARCIA, A. M. KHAN; Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: The *insular region (Brodmann, 1909)* (INS) (following the nomenclature proposed by Swanson LW (2015). *Neuroanatomical Terminology*, Oxford Univ. Press), plays a role in diverse functions such as interoception, gustation, emotional state recognition/regulation, social decision-making, social interaction, and aversive states. The INS contains *agranular insular areas (Swanson, 2018)* (AI) that include the *dorsal* and *ventral agranular areas (Krettek & Price, 1977)* (AId and AIv, respectively) and a *posterior agranular insular area (Krettek & Price, 1977)* (AIp). Although *endbrain (Kuhlenbeck, 1927)* (EB) regions involved in autonomic, limbic, and cognitive functions are known to be anatomically connected to the INS, AId and AIv connections with EB have not been compared and precisely mapped in a standardized reference atlas. Here, we characterized projections from the AId and AIv to EB subregions in a high-spatial resolution atlas (*Brain Maps 4.0 (BM4.0)*; Swanson, 2018, *J Comp Neurol*). The AId and AIv each received a cocktail of anterograde and retrograde tracers, *Phaseolus vulgaris* leucoagglutinin (PHA-L) and cholera toxin B subunit (CTb), respectively. The AId and AIv injection deposits mapped between *BM4.0* levels 9-13, and 9-12, respectively; with their centers

mapping to levels 10 and 11 (AP= +2.15 to +1.70 mm from bregma (β)) and level 10 (AP= +2.15 mm from β), respectively. PHA-L and CTb labeling were co-visualized by diaminobenzidine (DAB) and nickel-enhanced DAB reactions, respectively, in coronal brain sections. Whereas the AId displayed more projections dorsally in the *prelimbic area (Brodmann, 1909)* (PL) and *secondary somatomotor areas (>1840)* (MOs), projections from the AIv were more abundant ventrally in the PL, *infralimbic area (Rose & Woolsey, 1948)* (ILA), and *orbital region, (Rolando, 1831)* (ORB). Furthermore, the AId projections were distributed in various thalamic subregions and AIv projections mainly in the *mediodorsal thalamic nucleus (>1840)* (MD). Also, the amygdalar region had differences in dorsal and ventral expression except for the *lateral amygdalar nucleus (>1840)*. The *basolateral amygdalar nucleus (>1840)* (BLA) and *central amygdalar nucleus (Johnston, 1923)* (CEA) were heavily targeted by the AId, the AIv deposit resulted in robust PHA-L labeling in the *basomedial amygdalar nucleus (>1840)* (BMA) and *cortical amygdalar complex (Swanson, 2015)* (COX). Further analysis of anatomical connections of INS subregions will contribute to the design of functional experiments to understand its role in different behaviors.

Disclosures: L.P. Montes: None. **K. Negishi:** None. **M. Quintana:** None. **M. Garcia:** None. **A.M. Khan:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.04

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH GM127251
Eloise and Patrick Wieland Graduate Fellowship (KN)
NIH GM069621

Title: Standardized mapping and high-spatial resolution analysis of rostral and caudal prelimbic cortical connections in the endbrain (forebrain) of the adult male rat

Authors: *K. NEGISHI[1], L. P. MONTES[1], C. OLIVEROS[1], M. S. PONCE[1,2], Y. C. WELLS[1], A. M. KHAN[1];
[1]Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX; [2]Natl. Inst. of Dent. and Craniofacial Res., Bethesda, MD

Abstract: The *prelimbic area (Brodmann, 1909)* (PL) (following the nomenclature proposed by Swanson LW (2015). *Neuroanatomical Terminology*, Oxford Univ. Press) in rodents is a portion of the *cingulate region (Brodmann, 1909)* (CNG; “medial prefrontal cortex”) that is associated with a variety of functions including attention, executive control, and working memory. We have

previously compared connections of rostral and caudal parts of the PL using co-injected anterograde and retrograde tracers (*Phaseolus vulgaris* leucoagglutinin and cholera toxin subunit B, respectively). We used a standardized cytoarchitecture-based strategy that is the basis of a widely used reference atlas (*Brain Maps 4.0 (BM4.0); Swanson, 2018; J Comp Neurol*) to localize and compare histological data. Here, we expand on an earlier showcase of results by showing fully mapped datasets from the rostral pole of the *cerebral cortex (Swanson, 2000)* (CTX) to sections containing its caudal end (approx. +5.20 mm to −7.90 mm from Bregma). Among *cerebral nuclei (Swanson, 2000)* (CNU), pronounced differences were observed in the *accumbens nucleus (Ziehen, 1897-1901)* (ACB). Projections from the caudal PL densely targeted both ACB "shell" and "core" divisions whereas terminals from the rostral PL were restricted to the "core". Outputs to the nearby *olfactory tubercle (Calleja, 1893)* (OT) mainly originated from the caudal PL. The *caudoputamen (Heimer & Wilson, 1975)* (CP) received PL terminals adjacent to the *lateral ventricle (Vesalius, 1543)* (VL), with the rostral PL occupying a slightly more lateral field. The *hypothalamus (Kuhlenbeck, 1927)* (HY) received light projections from the rostral PL whereas the caudal PL innervated several hypothalamic structures, most of which were found in the *lateral hypothalamic area (Nissl, 1913)* (LHA). In the *thalamus (His, 1893)* (TH), PL connections were bidirectional. Rostral and caudal PL connections showed remarkably little spatial overlap despite having similar target structures. Caudal PL was strongly connected to the *mediodorsal thalamic nucleus medial part (>1840)* (MDm) whereas rostral PL mainly connected with its *central part* (MDc). The *paratenial nucleus (>1840)* (PT) connected with the caudal PL in its rostral part, but not its caudal extension, whereas rostral PL primarily connected with its caudal part. Overall, our structural work suggests that functional experiments involving the PL could produce variable effects depending on which rostrocaudal segment is targeted. Further anatomical and functional characterization of these circuits will help to refine their precision targeting and contribute to better division schemes for the rat CNG.

Disclosures: K. Negishi: None. **L.P. Montes:** None. **C. Oliveros:** None. **M.S. Ponce:** None. **Y.C. Wells:** None. **A.M. Khan:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.05

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251
HHMI PERSIST Program (JGR)

Title: Standardized mapping and high spatial-resolution analysis of the inputs and outputs of the accumbens nucleus "hedonic hotspot" in the male rat using Brain Maps 4.0

Authors: J. GUERRA-RUIZ[1], V. I. NAVARRO[2], K. NEGISHI[1], ***A. M. KHAN**[1];
[1]Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX; [2]Biol. Sci., The Univ. of Texas At El Paso, El Paso, TX

Abstract: The *accumbens nucleus (Ziehen, 1897-1901)* (ACB) (following the nomenclature proposed by Swanson LW (2015). *Neuroanatomical Terminology*, Oxford Univ. Press) is a major nexus of affect-based processing that is heavily connected to various regions which mediate emotional, reward and aversive responses. Some of these major regions have been neuroanatomically linked to the ACB using retrogradely transportable neuronal tracers deposited in various brain regions. Similarly, injections of anterograde tracers have been deposited into the ACB to determine if this region was sending information to other regions involved in affect-based processing. However, these various studies have not yet taken a detailed high spatial-resolution approach to create an atlas representation of the connections to and from this highly studied region. Here we use a combination of *Phaseolus vulgaris-leucoagglutinin* and cholera toxin B subunit to reveal the outputs and inputs, respectively, of the ACB. These connections were represented onto templates of a widely used reference atlas (*Brain Maps 4.0* (*BM4.0*); Swanson, 2018; *J. Comp. Neurol.*) spanning atlas levels 5-41. Preliminary results demonstrate reciprocating inputs and outputs from areas such as the *prelimbic area (Brodmann, 1909)* (PL), *lateral septal nucleus (Cajal, 1909-1911)* (LS), and *innominate substance (Schwalbe, 1881)* (SI). Major regions supplying inputs to the ACB include the *paraventricular thalamic nucleus (>1840)*, *paratenial nucleus (>1840)*, and *basolateral amygdalar nucleus (>1840)*, while major outputs from the ACB were found in the *anterior cingulate area (Brodmann, 1909)* (ACA), *lateral preoptic area (>1840)* (LPO), and *anteroventral preoptic nucleus (Simerly et al., 1984)* (AVP). Of these the regions, those that demonstrated the highest densities of tracer transport were found to be the PL, PVT, PT, BLA, and *subiculum (>1840)* (SUB). Thus far, these preliminary results are in line with previous studies that have placed tracers into other regions and observed transport to the ACB.

Disclosures: J. Guerra-Ruiz: None. **V.I. Navarro:** None. **K. Negishi:** None. **A.M. Khan:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.06

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH S10 OD016167
NIH grant P30 DK056338
NIH Grant R01DK109194

NIH Grant R56DK109194
Pew Charitable Trust awards 0026188
ADA #7-13-JF-61
USDA/CRIS grants 3092-5-001-059

Title: A hindbrain dopaminergic neural circuit prevents weight gain by reinforcing food satiation

Authors: *Y. HAN, G. XIA, Y. HE, Y. HE, M. FARIAS, Y. XU, Q. WU;
Baylor Col. of Med., Houston, TX

Abstract: The neural circuitry mechanism that underlies dopaminergic (DA) control of innate feeding behavior is largely uncharacterized. Here, we identified a subpopulation of DA neurons situated in the caudal ventral tegmental area (cVTA) directly innervating DRD1-expressing neurons within the lateral parabrachial nucleus (LPBN). This neural circuit potently suppresses food intake via enhanced satiation response. Notably, this cohort of DA cVTA neurons are activated immediately prior to the cessation of each feeding bout. Acute inhibition of these DA neurons prior to bout termination significantly suppresses satiety and prolongs the consummatory feeding. Activation of post-synaptic DRD1 LPBN neurons inhibits feeding, whereas genetic deletion of Drd1 within the LPBN causes robust increase in food intake and subsequent weight gain. Furthermore, the DRD1 LPBN signaling manifests the central mechanism in methylphenidate-induced hypophagia. In conclusion, our study illuminates a hindbrain DAergic circuit that controls feeding through dynamic regulation in satiety response and meal structure.

Disclosures: Y. Han: None. **G. Xia:** None. **Y. He:** None. **Y. He:** None. **M. Farias:** None. **Y. Xu:** None. **Q. Wu:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.07

Topic: F.09. Food and Water Intake and Energy Balance

Support: Supported by gifts for research from Cain-Stanley & Co.

Title: Evidence that $GABA_A$ receptors in the lateral septum mediate nocturnal feeding at the onset of the dark phase

Authors: *I. GABRIELLA[1], B. STANLEY[2];
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Abstract: Approximately 42.4% of US adults were afflicted with obesity from 2017 to 2018, a level which has steadily increased since 2000. Given the negative health impacts, understanding the neural substrates of overeating is a public health priority. Recent studies suggest that the lateral septum (LS) may be involved in eating control mechanisms. Previous research from our lab had shown that LS injections of $GABA_A$ and $GABA_B$ agonists rapidly elicit intense, dose-dependent feeding responses. At the same time, LS pretreatment with antagonists of GABA receptors decreased the elicited feeding. Together, these studies provide converging evidence that stimulation of GABA receptors in the LS can elicit eating. The current project investigated whether GABA receptors in the LS mediate nocturnal feeding at the onset of the dark phase of the light-dark cycle. Groups of adult male rats ($n \geq 11$/group) were microinjected with the $GABA_A$ receptor antagonist picrotoxin (0.1 μg / 0.3 μl), or the $GABA_B$ receptor antagonist, 2-OH saclofen (1.25 μg / 0.3 μl), or the aCSF vehicle (0.3 μl). Injections took place at the onset of the dark phase when rats eat spontaneously. Food and water intake was measured at 1, 2, 4, and 24 hours. Results showed that LS injection of the $GABA_A$ receptor antagonist picrotoxin produced an immediate and prolonged suppression of nocturnal feeding. In contrast, LS injections of the $GABA_B$ receptor antagonist 2-OH saclofen did not significantly reduce nocturnal eating. Water intake was unchanged in either condition, consistent with feeding behavior selective effects. These results suggest that activation of LS $GABA_A$ receptors but not $GABA_B$ receptors may be involved in nocturnal feeding. These findings may inform future research on pharmacological treatments for obesity or eating disorders.

Disclosures: I. Gabriella: None. **B. Stanley:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.08

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant HL084207
NIH Grant HL127673

Title: Conditional $RGS2^{MC4R}$ knockout mice show distinct metabolic phenotypes from whole-body RGS2 knockout mice

Authors: *Y. DENG[1], U. SINGH[1], K. SAITO[1], G. DENG[1], J. DICKEY[1], J. L. GROBE[2], H. CUI[1];
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Abstract: Obesity has become a major public health concern due to its high risk of developing life-threatening chronic diseases, such as hypertension and diabetes. While extensive evidence indicates that melanocortin-4 receptor (MC4R) and multiple other G protein-coupled receptors (GPCRs) signaling is critical for maintaining energy homeostasis, the intracellular regulatory components of these GPCRs affecting energy balance remain vague. Regulators of G protein signaling (RGS) proteins are key components of GPCR-Gα signaling by acting as GTPase-activating proteins (GAP) to turn off Gα signal transduction. RGS2, a member of the RGS family of proteins, has been implicated in metabolic control as conventional whole-body RGS2 knockout mice exhibit reduced body weight and disrupted adipogenesis. Our *in-situ* hybridization (ISH) analyses revealed that RGS2 is highly expressed in PVN where it co-expresses with MC4R, and that 24h food restriction significantly increased $RGS2^{PVN}$ expression. Based on these observations, we hypothesized that the lean phenotype observed in whole-body RGS2 knockout mice is due to loss of RGS2 in MC4R-expressing cells. Strikingly, however, conditional $RGS2^{MC4R}$ knockout mice showed significantly increased body weight compared to their littermate controls. Further analyses revealed that $RGS2^{MC4R}$ knockout mice have significantly altered body composition, increased body length and elevated fasting blood glucose levels. Interestingly, $RGS2^{MC4R}$ knockout mice did not exhibit disruption in eating behavior, except that males showed a moderately increased caloric intake when fed high-fat diet. Taken together, we found that $RGS2^{MC4R}$ knockout mice exhibit opposing metabolic phenotypes to whole-body RGS2 knockout mice, indicating that the role of RGS2 in energy homeostasis could be either tissue- or cell type-dependent, and that RGS2 might affects the physiology of MC4R-expressing neurons.

Disclosures: Y. Deng: None. **U. Singh:** None. **K. Saito:** None. **G. Deng:** None. **J. Dickey:** None. **J.L. Grobe:** None. **H. Cui:** None.

Poster

P648. Central Circuits for Feeding: Anatomy and Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P648.09

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant R01GM098932

Title: A neuronal network encoding food decisions under sensory conflict in Drosophila

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Abstract: Animals make everyday decisions about which foods to consume. Frequently such decisions are based on the comparison of subjective value of the available options after resolving

sensory conflict. For example, a hungry fly may choose a high calorie rotten fruit over a low calorie fresh option. Addressing such hierarchical adaptive decision-making has been difficult in human and mammalian models because of their cognitive and neuronal complexity and experimental restraints. To this end, we leveraged *Drosophila* fly gustation that comes equipped with a well described sensory taste system, a simpler yet rich behavioral repertoire, and high experimental tractability to understand the neuronal and molecular mechanisms underlying adaptive decision-making. *Drosophila* live around decomposing food matter and when hungry must balance obtaining essential nutrition with avoiding toxins, pathogens, etc. By virtue of their natural habitat, flies must weigh and integrate conflicting nutritive and harmful value of their food options before consumption. To emulate these naturalistic conditions, we employed a behavioral paradigm in which hungry freely walking flies sample and choose between different ratios of sweet and bittersweet foods. In this setup, we recorded the ingested food choice and the position of the flies on the food source. Concurrently, we manipulated cell-specific neuronal activity using optogenetics and RNAi knockdown while flies actively made decisions.
We found that when given a choice between a low calorie sweet option and a high calorie bittersweet option, hungry flies make trade-offs between the appetitive (sweet) and aversive (bitter) value of the food. In an optogenetic screen, we identified a neural ensemble that encodes adaptive food choice. This ensemble consists of upstream neuropeptidergic and dopaminergic neurons (DANs) that potentially encode and update internal metabolic state and food-related sensory information. Using receptor RNAi knockdown, we confirmed that these upstream inputs are relayed to a converging node in the neural sub-population called the fan-shaped body layer 6 (FBl6). FB is a *Drosophila* central brain region that is thought to be analogous to vertebrate basal ganglia, which is involved in mammalian decision making. Importantly, the dynamic neural activity in FBl6 neurons is context-dependent, changes with a fly's experience and hunger state, and represents the transient choice made by an individual fly.

Disclosures: P.F. Sareen: None. **L.Y. McCurdy:** None. **M.N. Nitabach:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.01

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Sexual differences in rodent hierarchical associative learning and reward-seeking

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Abstract: Reward learning is generally conceived as the binary association between some environmental stimuli and an ensuing rewarding outcome. However, animals can also engage in complex hierarchical associative learning in which certain stimuli (often contextual stimuli) acquire the ability to modify the predictive status of an ambiguous reward-paired cue (e.g. animals can learn that a cue results in reward if, and only if, it is encountered in a specific context). The behavioral and neurobiological bases of Binary and Hierarchical learning appear to be partially distinct, and alterations in the mechanisms of hierarchical associations have been proposed to contribute to several neuropsychiatric disorders (e.g. substance use disorder [SUD], post-traumatic stress disorder [PTSD]). However, studies into the behavioral and neurobiological basis of hierarchical associative learning have largely focused on male rodents. Here, we compared the learning and performance of male and female rodents in Binary vs. Hierarchical associative learning. Male and female Long Evans rats were trained in four different Pavlovian discrimination tasks. A first group of rats was trained in a context-dependent Pavlovian discrimination task in which the validity of two brief auditory tones (A and B) to signal a common reward was dependent upon the contextual visual background (Cx1 and Cx2, alternating every 2 min). Specifically, cue A was rewarded only in the Cx1, but not in the Cx2, while the opposite was true for cue B (Cx1: A+ / B-; Cx2: A- / B+). A second group was trained in a similar task, except that two distinct outcomes were used for the two rewarded trials (Cx1: A+/ B-; Cx2: A-/ B*). A third group was trained in a simple Pavlovian discrimination task in which one cue reliably signaled reward in both contexts, while the other cue was never paired with reward (Cx1: A+ / B-; Cx2: A+ / B-). Finally, for a fourth group both auditory cues were ambiguous predictors of reward regardless of the context (Cx1: A+- / B+-; Cx2: A+- / B+-). Critically, we observed sex-differences only in the first discrimination task (context-dependent discrimination with a single outcome), in which a majority (~75%) of male rats successfully learned the task, while only a minority (~35%) of females learned the task after 75 daily sessions. Further work will focus on identifying the behavioral and neurobiological mechanisms underlying these observed sex differences.

Disclosures: A.L. Maheras: None. **S. Peterson:** None. **J.I. Chavira:** None. **B. Wu:** None. **R. Keiflin:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Pritzker Neuropsychiatric Disorders Research Consortium
University of Michigan Endowment for Basic Sciences Accelerator Award
NIDA R21 DA052594

NIDA U01DA043098
Office of Naval Research (ONR) 00014-19-1-2149
T32DA07268

Title: Investigating the interaction between sex and vendor on the acquisition of goal- and sign-tracking behaviors in male and female rats

Authors: A. TURFE[1], S. R. WESTBROOK[2], S. A. LOPEZ[4], V. SANCHEZ FRANCO[5], *S. B. FLAGEL[2,3];
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Abstract: Cues in the environment become predictors of biologically relevant stimuli through associative learning. However, these cues can not only act as predictors, but also can be attributed with incentive value, gain excessive control over behavior, and lead to maladaptive behaviors characteristic of numerous psychopathologies. Rats that undergo Pavlovian conditioned approach (PavCA) training, in which the presentation of a discrete lever cue (conditioned stimulus, CS) is followed by the delivery of a food reward (unconditioned stimulus, US), will develop a conditioned response directed towards the lever-CS (i.e. sign-trackers), the food cup (i.e. goal-trackers), or vacillate between the two (i.e. intermediate responders). We have previously found that after just one PavCA session, corticosterone (CORT) plasma levels are higher in rats that become sign-trackers relative to those that become goal-trackers. Here, we assessed the effects of systemic CORT on the acquisition of sign- and goal-tracking behaviors in male and female rats, while examining whether vendor may play a role in these effects. Adult rats from either Charles River Laboratories or Taconic were used for these studies and rats from both vendors were run concurrently under identical experimental conditions. Rats underwent 5 sessions of PavCA training with 3 mg/kg CORT or vehicle injections (i.p.) administered 30 minutes prior to each session. The effects of CORT administration on sign-tracking and goal-tracking behaviors were assessed using several outcome measures, including the number of lever-CS vs. food cup contacts, the probability to approach the lever-CS vs. food cup, and the latency to approach the lever-CS vs. the food cup. CORT administration increased sign-tracking behaviors in male rats from Charles River, but not in those from Taconic. This interaction of treatment and vendor was present only in males, as there was not a significant effect of vendor, CORT treatment, or an interaction on the acquisition of sign-tracking or goal-tracking behaviors in female rats. Thus, the effects of systemic CORT administration on PavCA behavior differs between sexes and between rats from different vendors. We speculate that these effects are driven by differences in the distribution of behavioral phenotypes (i.e. sign-trackers vs. goal-trackers) between sexes and vendors. Ongoing studies are assessing whether differences in baseline CORT levels or glucocorticoid receptor expression between sexes and vendors might also contribute to these effects. These findings underscore the importance of reporting sex and vendor in scientific research and including them as critical variables of analysis.

Disclosures: A. Turfe: None. **S.R. Westbrook:** None. **S.A. Lopez:** None. **V. Sanchez Franco:** None. **S.B. Flagel:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH T34-GM118212 MARC U*STAR Program
Gayle & Ben Batey Neuroscience Fund

Title: The effects of diet and environment on sucrose reinforcement and spatial learning in rats

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Abstract: In humans, it is well known that a diet high in fat and sucrose can lead to negative health outcomes including obesity, diabetes, and cardiovascular disease. Less known are the effects of a high fat diet and sucrose on behaviors such as reward-seeking and learning. In the context of brain function, recent data in rodents have shown that both high-fat diets and sucrose can alter dopamine function in the mesolimbic reward circuitry providing clues as to how both may affect behavior. The aim of our research was to investigate the effect of a high-fat diet containing sucrose (Western Diet) on operant responding and spatial learning. Additionally, we were interested in the known positive effects of environmental enrichment on both behaviors. Rodents were fed a Western diet containing sucrose (45% fat and 6% sucrose) or a low-fat diet lacking sucrose (standard chow, 14% fat no sucrose) for 30 days while housed in either enriched or standard housing. After 30 days, rats were tested for operant responding for 10% sucrose solution for 7 days. Daily sessions were 60 min in duration, during which snout pokes to the active alternative resulted in presentation of 100ul of sucrose according to a Fixed Ratio 1 (FR1) schedule of reinforcement, which was increased to an FR5 schedule on Day 3. Rats fed a Western diet earned significantly fewer sucrose rewards than the low-fat no sucrose group. After 7 days of 10% sucrose testing, operant responding for 4 additional concentrations of sucrose (0.1, 0.3, 1.0, 3.0%) were measured in a once daily FR5. Rats fed a Western diet earned significantly more sucrose rewards at 0.1% and 0.3% concentrations compared to their low-fat, no sucrose counterparts. Taken together, these data suggest the Western diet may produce a leftward shift in the concentration response to sucrose solution. Enrichment produced no significant changes in sucrose reinforcement. Following operant testing, rats were then tested for spatial learning (8-arm maze) for 8 days. Rats fed a Western diet significantly committed more errors as compared to their low-fat no sucrose counterparts. However, enrichment ameliorated the negative effects of the Western diet. Overall, the western diet adversely affected both operant and learning behavior as shown by the leftward shift in the concentration response which may

indicate an increased sensitivity to sucrose, and the increased errors in the 8-arm maze. Interestingly, enrichment was able to ameliorate the negative effects of the Western diet in spatial learning but not in reward-seeking. These data may have implication for humans, suggesting a link between chronic early Western diet intake and future reward-seeking and learning.

Disclosures: S. Winchester: None. **L. Herrera:** None. **J. Pulido:** None. **F. Gomez:** None. **A.M. Gancarz-Kausch:** None. **I.C. Sumaya:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Pritzker Neuropsychiatric Disorders Research Consortium
University of Michigan Endowment for Basic Sciences Accelerator Award
NIDA R21 DA052594
NIDA U01DA043098
Office of Naval Research (ONR) 00014-19-1-2149
T32DA07268
National Science Foundation Graduate Research Fellowship DGE 1256260

Title: Transgenic rat strains differ in attribution of incentive value to reward-associated cues

Authors: *S. R. WESTBROOK[1], A. G. IGLESIAS[2], S. A. LOPEZ[5], H. E. DAVIES[3], F. LI[1], H. AKIL[1,4], K. DEISSEROTH[6], J. P. HERMAN[7], S. B. FLAGEL[1,4];
[1]Michigan Neurosci. Inst., [2]Neurosci. Grad. Program, [4]Psychiatry, [3]Univ. of Michigan, Ann Arbor, MI; [5]Oregon Hlth. & Sci. Univ., Portland, OR; [6]Bioengin & Psych, Stanford Univ. Dept. of Psychology, Stanford, CA; [7]Dept Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Transgenic rat strains are becoming increasingly available and popular for use in neuroscience research. However, the behavioral phenotypes of these transgenic rat strains are often not studied or overlooked in favor of their utility for probing neural mechanisms. Here, we compared the tendency of three transgenic rat strains—glucocorticoid receptor (GR)-CRISPR, tyrosine hydroxylase (TH)-Cre, and Orexin-Cre—to attribute incentive value to a reward-associated cue in a Pavlovian conditioned approach (PavCA) task. All three transgenic strains were bred in-house at the University of Michigan. GR-CRISPR rats have a Sprague Dawley background, while TH-Cre and Orexin-Cre rats have a Long Evans background. Adult rats were exposed to a PavCA task for 5 sessions, during which an illuminated lever (conditioned stimulus,

CS) was extended for 8 seconds before it was retracted, and a food pellet (unconditioned stimulus, US) was delivered into an adjacent food cup. Each session consisted of 25 lever-food (CS-US) pairings. Upon Pavlovian learning, rats developed a conditioned response directed towards the lever-CS (sign-trackers, STs), the food cup-US (goal-trackers, GTs), or they vacillated between the two (intermediate responders, IRs). Rats were classified as STs, GTs, or IRs based on a composite PavCA index that incorporates the numbers of contacts with, the probability to approach, and the latency to approach the lever vs. the food cup during lever presentations. We found that the three transgenic strains differed in their distribution of these behavioral phenotypes. The GR-CRISPR strain is heavily skewed towards STs (87%) with zero GTs to date after 6 litters across F1 and F2 generations. The TH-Cre strain is also skewed towards STs (72%). The Orexin-Cre strain shows a more even distribution between STs (41%), IRs (39%), and GTs (20%), which is similar to what we have observed in outbred rat strains (~33% each). Importantly, these differences in transgenic strains are not due to breeding protocols, as all were bred in-house with similar procedures and precautions to minimize inbreeding. Nor can these findings be explained by background strain, as both TH-Cre and Orexin-Cre rats have a Long Evans background, yet the TH-Cre line is skewed towards STs. These findings demonstrate that we should not assume the distribution of behavioral phenotypes in transgenic rat strains is the same as outbred strains or as other transgenic strains based on genetic background or breeding factors. Further, these findings have significant implications for the use of transgenic rat strains and emphasize the need to determine behavioral phenotypes prior to probing neural mechanisms.

Disclosures: S.R. Westbrook: None. **A.G. Iglesias:** None. **S.A. Lopez:** None. **H.E. Davies:** None. **F. Li:** None. **H. Akil:** None. **K. Deisseroth:** None. **J.P. Herman:** None. **S.B. Flagel:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 1F99NS120544-01
R01DA044960

Title: Effects of chemogenetic inhibition of the ventral hippocampus to nucleus accumbens projection on sign- and goal-tracking behavior

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Abstract: Reward-associated cues can acquire excessive motivational value, resulting in maladaptive behaviors like those linked to addiction and relapse. Individual differences in Pavlovian conditioned approach (PCA) behavior can be used to detect increased sensitivity to reward cues, an identified risk factor for addiction. "Sign-tracking" rats (ST) reliably approach a reward-associated cue (i.e., lever) and interact with it, while "goal-tracking" rats (GT) direct their conditioned behavior away from the cue and towards the site of impending food reward (i.e., food cup). Compared to GT, ST not only use the cue as a predictor of the reward, but also attribute it with incentive salience, indicating that the cue becomes rewarding in and of itself. Glutamatergic transmission in the nucleus accumbens (NAc) — a critical region of the motive circuit - is necessary for the development of sign-tracking, but it remains unclear which glutamatergic afferents to the NAc play this functional role. The NAc is densely innervated by the ventral hippocampus (vHPC), and past work in our lab showed that lesions of the vHPC attenuate the acquisition of sign-tracking. However, whether this effect is exerted via direct modulation of the NAc is unclear. We explored the hypothesis that the vHPC-NAc projection plays a functional role in determining the ST/GT phenotype. We used an *in vivo* dual-vector approach by injecting Cre recombinase into the NAc and an inhibitory Cre-dependent, Gi-coupled *designer receptor exclusively activated by designer drugs* (*DREADD*) into the vHPC to selectively target the vHPC-NAc projection. After 5 weeks of incubation, rats were subjected to 6 daily sessions of PCA with clozapine-N-oxide (CNO; 3mg/kg, i.p.) or vehicle (6% DMSO, i.p.) on board, followed by a cross-treatment test session. We found that inhibition of the vHPC-NAc projection had no significant effect on the acquisition of sign- and goal-tracking behavior. However, after disinhibition of the vHPC-NAc during the cross-treatment test session, rats classified as ST, showed a significant decrease in sign-tracking compared to their last acquisition session, suggesting that chronic vHPC-NAc inhibition affected their expression/performance of sign-tracking. GT's behavior in the test session remained unaffected. Our data suggests that the vHPC-NAc projection might play a selective role in the performance of sign-tracking behavior in ST. More experiments need to be performed to further dissect this role and shed light on the neurocircuitry responsible for biasing ST/GT behavior.

Disclosures: C.E. María-Ríos: None. **F. Czesak:** None. **C. Groves:** None. **P. Campus:** None. **J.D. Morrow:** None.

Poster

P663. Acquisition II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P663.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSF Grant 1844144

Title: Using Pavlovian-Instrumental Transfer to Test a Modified Law of Effect

Authors: *H. P. DE GUZMAN, I. ENRIQUEZ, A. P. BLAISDELL;
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Abstract: During the extinction of a learned response, increases in the variability of the response (e.g., temporal, spatial) have been observed. Edward Thorndike's Law of Effect (LOE), while a popular model of stimulus-response (S-R) learning processes, fails to account for many observations during extinction, such as increased behavioral variability. A modified Law of Effect (MLOE) was recently proposed to expand the explanatory power of the LOE, adding several postulates to the original tenet of the LOE which mimic real world neural processes such as mutual inhibition between excitatory neurons. To test the MLOE, a Pavlovian-Instrumental transfer (PIT) procedure was administered to rats. In Experiment 1, 16 female rats were first administered Pavlovian conditioning; they were presented with a continuously reinforced cue (CRF; 100% probability) and a partially reinforced cue (PRF; 25% probability) on separate trials which were paired with delivery of sucrose pellets. Then, during instrumental training, rats learned to make a lever press response for sucrose pellet delivery. A final PIT test presented each conditioned stimulus (CS) during extinction of instrumental lever pressing. We assessed variability using the coefficient of quartile variance (CQV) for both lever press durations and lever press inter-response times (IRT) during the PIT test. Because each CS had never been present during instrumental training, the MLOE predicts no difference in lever press variability elicited by the Pavlovian cues. We found instead that variability was lower during the CRF cue than the PRF cue in both lever press duration ($t(24) = 2.474$, $p = 0.021$) and IRT ($t(21.937) = 2.446$, $p = 0.023$), which supports the Law of Expect (LOX), that is, as outcome expectancy decreases, behavioral variability increases. Due to the use of pairing a single lever with a single reward during instrumental training, it is unclear whether the transfer effects were due to specific or general PIT, each of which is mediated by different neural mechanisms. The literature suggests this design produces general PIT. Thus, in Experiment 2, we incorporated additional food reward outcomes and paired two out of three of those outcomes with two different levers during instrumental training, theoretically allowing us to observe specific PIT. Results allowed us to discriminate between predictions of MLOE, which accounts for specific PIT in terms of S-R processes, and the LOX, which accounts for general PIT in terms of outcome expectation.

Disclosures: H.P. De Guzman: None. **I. Enriquez:** None. **A.P. Blaisdell:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.01

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Multi-site electrophysiological recordings reveal synchronic activity in the basal ganglia during sucrose consumption

Authors: *E. CASEY[1], A. KRAVITZ[1,2,3];
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Abstract: Obesity is a growing public health concern due to its increasing prevalence and because it is associated with a variety of disabling and fatal health conditions. Though several factors account for the development of obesity, caloric intake above the physiological needs is one of its major causes. Despite animal research has revealed several circuits and cell types that mediate feeding, the actual contribution of each one to the development of obesity is still unclear. Hedonic motivation for highly palatable foods drives humans and animals to overeating, and evidences from humans and animal research indicate that the NAc is a central regulator of hedonic feeding and is also a major site of dysfunction in obesity. Then, with the goal of finding global activity signatures for hedonic feeding that could play a role in the development of obesity, we recorded local field potentials simultaneously in the NAc and its three major inputs (the prefrontal cortex (PFC), the basolateral amygdala (BLA) and the ventral hippocampus (vHip)) while mice received intra-oral infusion of sucrose solutions. A principal component analysis revealed an increase in the power of the low gamma band in the PFC, BLA and NAc, locked to the liquid infusion. Moreover, synchronicity at the low-gamma band between the PFC, NAc and BLA significantly increased during infusions, although this signaling was insensitive to differences in concentration of sucrose. Though this signaling does not seem to reflect the hedonic value of the liquid infused, we evaluated if low gamma synchronic activity can predicts variations in weight gain. Our data suggest that the level of synchronicity between the PFC and the NAc at the low gamma band correlates with the weigh gained after one week of high fat diet. Altogether, our preliminary results points that synchronic oscillatory activity at the low gamma band between the NAc and its inputs is involved in the signaling of food delivery and may predict weight gain.

Disclosures: E. Casey: None. **A. Kravitz:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: TCU SERC G-180614
TCU SERC UG-180534

Title: Chemogenetic manipulations of the globus pallidus externus in frustration: Selective effects during the early stages of reward downshift

Authors: S. GUARINO, *C. W. HAGEN, *M. R. PAPINI;
Texas Christian Univ., Fort Worth, TX

Abstract: The role of the globus pallidus externus (GPe) in the adjustment to reward downshift and open field (OF) activity was studied using designer receptors exclusively activated by designer drugs (DREADDs). Rats exposed to unexpected downshifts of sucrose concentrations exhibit significant suppression of licking response to the downshifted sucrose solution relative to unshifted controls always given access to the same sucrose solution. In the current study, groups of rats received bilateral infusion of the inhibitory hM4D(Gi) or excitatory hM3D(Gq) designer receptors into the GPe. Inhibition of the GPe using the DREADD activator clozapine N-oxide (CNO) to activate the hM4D(Gi) receptors led to reduced licking in early 32-to-2% sucrose downshift sessions, but not in late downshift sessions. By contrast, excitation of the GPe using CNO to activate the hM3D(Gq) receptors led to increased licking in early 12-to-2% downshift sessions, but not in late downshift sessions. These chemogenetic manipulations had no detectable effects on OF activity. Another set of experiments, using the same 32-to-2% sucrose downshift for inhibitory DREADDs and an 8-to-2% sucrose downshift for excitatory DREADDs, replicated the selective effects of neural manipulation in the early downshift sessions, as well as the lack of detectable effects in late downshift sessions and in the OF task. These results are the first to show opposite effects of chemogenetic inhibition vs. excitation of GPe neurons on behavior during a reward downshift event. Furthermore, these effects were observed during the early stages of reward downshift, when the emotional response to reward loss is at its peak, without affecting the late stages of reward downshift, when recovery of consummatory behavior had occurred. These effects occurred in the absence of any evidence of motor alterations after either inhibitory or excitatory DREADD activation in the OF. This research is the first to demonstrate a role of the GPe in frustrative nonreward, encouraging further investigation of potential functions for other basal ganglia structures in frustration.

Disclosures: S. Guarino: None. **C.W. Hagen:** None. **M.R. Papini:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: S.X.C. supported by Canada Research Chair (CRC) (Grant no. 950-231274)
S.X.C. supported by Natural Sciences and Engineering Research Council of Canada (NSERC) (Grant no. 05308)

C.L. supported by OGS/QEII scholarship
E.H. supported by a NSERC graduate scholarship
R.N supported by NSERC (Grant no. 06972)

Title: Cell-type specific responses to associative learning in the primary motor cortex

Authors: *C. LEE[1], E. HARKIN[1], R. NAUD[1,3,2,4], S. X. CHEN[1,3,4];
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Abstract: The primary motor cortex (M1) is a critical site for movement initiation and motor learning. Intriguingly, it has been demonstrated that neurons in M1 also show reward-related activity, possibly to facilitate reward-based learning of new movements. However, whether reward-related signals are represented among different cell types in M1, and whether their response properties change after cue-reward conditioning remains unclear. Here, we performed longitudinal *in vivo* two-photon Ca^{2+} imaging to monitor the activity of different neuronal cell types in M1 of adult mice engaged in a classical conditioning task. Our findings reveal that most of the major neuronal cell types in M1 show robust but differential responses to both cue and reward stimuli, and their response properties undergo cell-type specific modifications following associative learning. PV inhibitory neuron responses became more reliable to the cue stimulus, while VIP inhibitory neuron responses became more reliable to the reward stimulus. Pyramidal neurons showed robust responses to novel reward but this diminished following conditioning. Lastly, SOM inhibitory neuron responses emerged and became more reliable to both conditioned cue and reward stimuli after conditioning. These observations suggest that cue- and reward-related signals are represented among different neuronal cell types in M1, and the distinct modifications they undergo during associative learning could be essential in triggering different aspects of local circuit reorganization in M1 during reward-based motor skill learning.

Disclosures: C. Lee: None. **E. Harkin:** None. **R. Naud:** None. **S.X. Chen:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant U19NS113201
NIH Grant RO1NS108740
Simons Collaboration on Global Brain
Promotion of Science, Japan Science and Technology Agency
Brain Mapping by Integrated Neurotechnologies for Disease Studies

AMED JP20dm0207069

Title: A gradual backward shift of dopamine responses during associative learning

Authors: *R. AMO[1], S. MATIAS[1], A. YAMANAKA[2], K. F. TANAKA[3], N. UCHIDA[1], M. WATABE-UCHIDA[1];
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Abstract: Multiple lines of evidence suggest that the phasic activity of midbrain dopamine neurons convey temporal difference (TD) prediction error, a teaching signal developed in reinforcement learning frameworks. A fundamental prediction of this model is that dopamine activity should resemble the dynamics of TD errors during the learning process. However, whether this similarity holds true during learning remains elusive. Specifically, a class of TD learning models using a temporal representation based on sequential activation of neurons (complete serial compound) predicts that the error signal gradually shifts backward in time from reward delivery to a reward-predictive cue. Still, such a shift has not been demonstrated experimentally, putting into question its existence, and the TD explanation of dopamine phasic activity. In this study, we show that a gradual backward shift in peak dopaminergic activity occurs over trials during associative learning. Specifically, to detect a small activity "bump" between cue and reward on a trial-by-trial basis, we used optical methods that integrate signals from a large population of neurons (fiber fluorometry) and across time (i.e. slow measurements using calcium indicator or dopamine sensor). To temporally resolve slow signal modulations, we included a relatively long delay between the cue and the outcome (e.g. 3 sec). As a result, we reliably observed a gradual backward shift of dopamine signal in the ventral striatum (VS) during learning of odor-reward association, especially in naïve mice ($p = 0.16 \times 10^{-4}$, $n = 7$ mice). This shift was also observed in well-trained mice ($p = 0.65 \times 10^{-3}$, $n = 5$ mice). A gradual backward shift of activity was also observed using fiber fluorometry of calcium signals measured from dopamine neuron soma in the ventral tegmental area (VTA). To resolve single neuron activity, we next performed 2-photon calcium imaging in the VTA. We observed a gradual backward shift of activity in single dopamine neurons although the extent and speed of the shift varied across neurons. Our modeling confirms that integrating signals over time using slow measurements can indeed facilitate the detection of such small and time-spread activity. These results establish that, at least in some conditions, a gradual backward shift of dopamine responses, a long-sought link between TD learning and dopamine activity, can be observed during associative learning. These shared dynamics of TD error and dopamine activity provide a critical evidence supporting the TD account of dopamine signals.

Disclosures: R. Amo: None. **S. Matias:** None. **A. Yamanaka:** None. **K.F. Tanaka:** None. **N. Uchida:** None. **M. Watabe-Uchida:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 1ZIAEY000415

Title: Lateral habenula neurons encode step-by-step changes of reward prediction

Authors: *H. LEE[1], O. HIKOSAKA[2];
[2]Lab. Sensorimotor Res., [1]Natl. Eye Inst., Bethesda, MD

Abstract: Changes of prediction play critical role in sequential goal-directed behaviors (e.g., Chess and Go games). In the brain, lateral habenula (LHb) and dopamine neurons have been widely studied to encode value of reward outcome compared with their previous prediction (Matsumoto and Hikosaka, 2009). To reach a reward outcome, we often need appropriate multiple goal-directed behavior step-by-step. However, brain mechanisms have been poorly studied in multiple steps of goal-directed behaviors. Here we hypothesized that LHb play an important role in sequential goal-directed behaviors with encoding step-by-step changes of reward prediction. Therefore, we devised triple-step saccade task consisted of the series of reward-oriented events (environment, action cue, and object), each of which changed reward prediction multiple times before reaching a final outcome of a trial. We found that LHb neurons represent changes of reward prediction, selectively between before and after each event. At each event, LHb activity changed sequentially in multiple contexts according to the strength of reward prediction errors. This finding suggests that LHb can play a critical role to calculate reward prediction error to control action sequence and action skills in multiple steps of sequential goal-directed behaviors.

Disclosures: H. Lee: None. **O. Hikosaka:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH F31 MH125542-01A1

Title: Dopaminergic control of the explore-exploit tradeoff

Authors: *L. VARGAS, M. V. FUCCILLO;
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Abstract: In order to effectively make decisions, individuals must use prior experiences to balance choices that have a known positive outcome (exploitation) and choices that sample alternatives to better estimate the comparative value of the current selection (exploration). Altered regulation of these fundamental behavioral states is essential for survival and may underlie executive function defects in neuropsychiatric disorders such as autism, obsessive compulsive disorder, Tourette syndrome and addiction. The dorsal striatum is an important brain region for decision-making and action-selection. Recent literature suggests that dopamine may mediate the explore-exploit tradeoff by modulating activity in striatal direct pathway and indirect pathway neurons. According to this model, exploration would be characterized by increased dopamine, increased activity in direct-pathway neurons, and therefore a greater number of active ensembles representing different potential actions. Alternatively, exploitation would be characterized by reduced dopamine, reduced activity in direct-pathway neurons, and therefore a smaller number of active ensembles. To study how dopamine mediates the balance between exploration and exploitation, we generated a novel 3-alternative choice task for mice. Mice gain access to three nosepoke ports via a touchscreen-activated doorway. Each port has a different probability of reward that is shuffled between blocks of trials. The animals must continually learn the value of the ports by sampling ports and evaluating outcomes. We employed three different classifiers of choice behavior from the literature to define exploration and exploitation: consecutive choice, hidden Markov models, and softmax-based reinforcement learning models. To record in vivo dorsal striatal dopamine signals during task execution, we injected the GRAB-DA sensor in either dorsomedial (DMS) or dorsolateral (DLS) striatum. We found that there was robust modulation of the dopamine signal as mice engaged in the task, particularly in the pre-choice period when mice are standing on the touchscreen. Furthermore, we found these pre-choice signals were differentially modulated depending on whether the current choice was exploring or exploiting. These data will provide important experimental tests of theories implicating dopamine in explore-exploit behaviors and will generate a framework for testing causal contributions of dopamine to decision-making strategies.

Disclosures: L. Vargas: None. **M.V. Fuccillo:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NEI EY017271

NIH EY030226

Title: Fef neurons signal surprise separately from value

Authors: *M. SHTEYN[1,2], C. R. OLSON[1,2];
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Abstract: Our previous research has demonstrated that neurons in the macaque frontal eye field (FEF)respond to visual cues with a strength that depends both on the value of the reward they predict and onhow surprising the reward is. The effects related to value and surprise might reflect dependence ofneuronal activity on a single latent factor, for example the capture of attention, or might beindependent. The possibility that the signals are independent is supported by the observation in ourprevious studies that the timing of signals related to value and surprise is different. Here, we approachthe issue directly by asking whether, across the neuronal population, signals related to value andsurprise are dissociable. In a task requiring central fixation, macaque monkeys viewed sequences oftwo visual cues followed by the delivery of a reward. The leading image predicted with highprobability ($p = 0.83$) the delivery of either a large or small reward at the end of the trial. The trailingimage indicated with certainty that the ensuing reward would be large or small. The informationconveyed by the trailing image usually confirmed ($p = 0.83$), but occasionally violated ($p = 0.17$), theprediction conveyed by the leading image. Overall, neurons responded more strongly to a trailingimage that violated an expectation set by the leading image than to a trailing image that confirmed anexpectation set by the leading image. Firing was enhanced for outcomes that were both better thanexpected and worse than expected. However, the dynamics of the response depended on whether thesurprise was good or bad. In response to good surprise, FEF neurons enhanced their firing rate in boththe early and sustained components of their visual response. In response to bad surprise, FEF neuronssuppressed their firing rate in the early component of their response, but enhanced their firing rate inthe sustained component of their response. Upon measuring the correlation across neurons between thestrength of the value signal and the strength of the surprise signal, we found that each variableaccounted for only a small fraction of the explainable variance in the other. We conclude that value-related and surprise-related signals in the FEF are dissociable not only with respect to their dynamicsbut also with respect to the populations of neurons carrying them.

Disclosures: M. Shteyn: None. **C.R. Olson:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Dartmouth startup funds to MVDM
Dartmouth Fellowship to JMG

Title: Contextual gating of motivationally-relevant stimuli in the mouse nucleus accumbens

Authors: *J. M. GMAZ, M. A. A. VAN DER MEER;
Dartmouth Col., Hanover, NH

Abstract: The nucleus accumbens (NAc) is an important contributor to the motivational control of behavior, acting directly through output pathways involving brainstem motor nuclei ("limbic-motor interface") and indirectly through return projections within cortico-striatal loops. Accordingly, neural activity in the NAc is thought to track fundamentally value-centric quantities such as current or future expected reward, reward prediction errors, the value of work, opportunity cost, and approach vigor. However, in complex, dynamic behavioral tasks, lesions or inactivations of the NAc lead to deficits that are not straightforward to explain from a purely value-centric perspective. In addition, prominent corticolimbic inputs suggest that the NAc has access to non-value signals that would be expected to not only inform its function but help shape its neural activity. This raises the question of if, and how, non-value signals are encoded in NAc, and if found, how such a signal relates to classical value correlates.
To address this issue, we trained 4 mice to perform a biconditional discrimination task using odor cues, in which two different "context" cues determine whether a subsequent "target" cue will be rewarded. Thus, in context O1, O3 but not O4 is rewarded, whereas in context O2, O4 but not O3 is rewarded. We recorded ensembles of NAc neurons and tested whether there is coding of the (reward-independent) context cues at the single cell and population level. We found coding for context cues in 26% of all recorded units, that could not be attributed to differences in the perceived values of the cues.
Next, we sought to determine the relationship between this context signal and classical value coding, using contemporary population analysis tools to test if context coding can be used to inform subsequent value-related processing of target cues. We found that the population level context signal occupied a neural subspace orthogonal to classic value representations, suggesting that both can be read out without interfering with each other. Despite this independence, the context signal could explain a significant proportion of variance in the subsequent value signal in response to a given target cue.
Together, the finding of clear non-value correlates suggests a revision of the value-centric account of NAc neural activity, encouraging future work to explore the precise computational role of these signals. Furthermore, the functional link at the population level between the context signal and value signal supports a circuit-level gating model for how the NAc contributes to behavioral flexibility and provides a novel population level perspective from which to view NAc computations.

Disclosures: J.M. Gmaz: None. **M.A.A. van der Meer:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.09

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Selectivity of pavlovian to instrumental transfer is regulated by dorsal raphe projections to the nucleus accumbens

Authors: D. N. TAPP, C. M. BODNER, Z. PLATOW, E. BOGDANSKI, G. PETRYK, *M. S. MCMURRAY;
Psychology, Miami Univ., Oxford, OH

Abstract: Learning about reward predictive cues and responding for rewards is an adaptive component of motivated behavior. Previously learned reward predictive cues can influence the vigor of ongoing operant responding through a process called pavlovian to instrumental transfer (PIT). For example, a previously learned drug predictive cue can increase the intensity of on ongoing operant response, such as lever pressing, even when the lever does not result in the delivery of a drug reward. This process is fundamentally disrupted during substance use disorders. Thus, identifying the neural systems involved is key to developing effective treatments. The mesocorticolimbic dopamine system plays a critical role in pavlovian cue learning, operant responding, and PIT. The dorsal raphe nucleus (DRN) projects to the mesocorticolimbic system at both the level of the cell bodies in the ventral tegmental area (VTA) and the primary projection site in the nucleus accumbens (NAc). Thus, DRN activity may regulate cue learning and responding by influencing neural activity within the VTA and NAc. Recent work established that activation of projections between the DRN and the VTA is reinforcing and promotes dopamine release into the NAc. However, the role of DRN projections to either the VTA or the NAc in PIT has yet to be established. Here, we utilized an excitatory chemogenetic approach (DREADDs) in a rodent model to determine how DRN projections influence cue learning, operant responding, and PIT. Following recovery from surgery, animals completed ten sessions of pavlovian conditioning, in which three auditory cues predicted the delivery of three unique food rewards. Next, animals completed six sessions of random ratio training, in which each of two levers predicted the delivery of two of the previously paired rewards. Finally, animals were tested for PIT on two separate days using an extinction task where one lever was available and pavlovian cues were played, but no rewards were delivered. Excitation of DRN to NAc projections produced a selective suppression of PIT responses, in which animals were less likely to make lever presses during cues that had never been paired with the operant response. DRN to VTA excitation did not enhance or suppress PIT responses compared to control subjects. These results suggest that selective activation of DRN projections to the NAc may alter the generalizability of reward-predictive cues. Further, these results suggest that the DRN projections to the NAc may warrant further investigation in the context of addiction and impulse control disorders.

Disclosures: D.N. Tapp: None. **C.M. Bodner:** None. **Z. Platow:** None. **E. Bogdanski:** None. **G. Petryk:** None. **M.S. McMurray:** None.

Poster

P665. Neural Systems II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P665.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Intramural Research Grant

Title: One group of dopamine neurons is sensitive to peripheral visual input before saccade

Authors: *G. COSTELLO, O. HIKOSAKA;
Natl. Inst. of Health/NEI, Bethesda, MD

Abstract: Midbrain dopamine (DA) neurons are known to exhibit short-latency phasic activity to conditioned stimuli. The traditional hypothesis suggests that this phasic response signal the reward predictions errors (RPE), but a more recent hypothesis suggests that this response reports the saliency of the stimuli. This study proposes to test both the reward prediction hypothesis and the stimulus salience-based alternative hypothesis of DA function. We recorded 96 DA cells in two monkeys during a broad spectrum of behavioral tasks, including Pavlovian conditioning with appetitive and aversive associations. Consistent with previous findings from our lab the rostral-ventral-medial region (rvmSNc) contains DA neurons that are activated by appetitive conditioned stimuli and are suppressed by aversive conditioned stimuli (Value Cells). Conversely, DA neurons in the caudal-dorsal-lateral region (cdlSNc) are activated by both appetitive and aversive conditioned stimuli (Salience cells). More importantly our analysis of the response of DA neurons to reward-predicting stimuli reveled two distinct timescales. In the salience cells there was an early phasic burst around 90ms which is rapidly followed by a second burst at 140ms. In contrast in the value cells, we found only the second burst of activity at 140ms. More interestingly when the DA activity is aligned on the saccade, in the salience cells the early phasic burst was presaccadic while the late second burst was post saccadic. In the value cells the single burst of activity was post saccadic. We suggest that the early phasic burst represent the stimulus driven saliency component while the second burst after the saccade initiation represent the value based and may code for RPE.

Disclosures: G. Costello: None. **O. Hikosaka:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.01

Topic: G.03. Motivation

Support: NIH T32DA007234-34
NIH T32NS105604-02

Title: Ventral pallidum projections to the ventral tegmental area encode reward outcome, but not the incentive motivational value of cues

Authors: *D. PALMER, A. SCOTT, M. WEBERG, I. LIN, J. RICHARD;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Reward-predictive sensory cues contribute to escalation and reinstatement of maladaptive seeking behavior in addiction. Reward cues exert their influence through dissociable psychological mechanisms, including incentive motivation and conditioned reinforcement. Ventral tegmental area (VTA) dopamine circuits have been implicated in both the motivating and reinforcing effects of cues. The ventral pallidum (VP) is a major input to the VTA and common node among preclinical models of relapse. Previous work demonstrated that VP projections to the VTA are necessary for cue-induced reinstatement of cocaine seeking, yet the associative processes by which these projections (VP→VTA) promote seeking are unclear. Given prior evidence that VP neurons encode the incentive motivational value of cues, we hypothesized that VP→VTA neurons respond to reward-predictive cues and encode the vigor of seeking behavior. To test this hypothesis, we used fiber photometry to measure VP→VTA calcium activity in female Long Evans rats (n=9) during a discriminative stimulus (DS) task. To target VP→VTA specifically, we injected a virus expressing a cre-dependent genetically encoded calcium sensor (GCaMP6f) into the VP and a retrograde virus expressing Cre into the VTA. Optical fibers were implanted over the VP to collect bulk fluorescence signals. Rats were then trained in the DS task, in which one auditory discriminative stimulus (DS) signals availability of 10% sucrose, contingent on rats' port-entry. A neutral stimulus (NS) is also presented which is never associated with reward availability. Contrary to our hypothesis, we did not observe VP→VTA responses to DS cue onset. Moreover, post-DS calcium signal did not predict port entry latency. Instead, we observed robust activity following port-entry on DS trials, during the time of sucrose consumption. These results suggest that VP→VTA encode outcome value of reward but not the incentive motivational value of cues. Our findings suggest that previous effects of VP→VTA manipulations on cue-induced reinstatement may have been due to the contributions of this projection to conditioned reinforcement, not invigoration, by the drug cue.

Disclosures: D. Palmer: None. **A. Scott:** None. **M. Weberg:** None. **I. Lin:** None. **J. Richard:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.02

Topic: G.03. Motivation

Support: R00AA025384

Title: Investigating ventral pallidal encoding of expected outcome value

Authors: *A. SOOD[1], J. M. RICHARD[2];
[2]Dept. of Neurosci., [1]Univ. of Minnesota, Minneapolis, MN

Abstract: Disruptions in goal-directed decision-making are a core component of several neuropsychiatric conditions, including addiction. Goal-directed behavior relies on accurate mental representations of future outcomes and their expected value. An understanding of the neural mechanisms underlying expected value encoding within the brain reward centers is key for developing effective long term treatments for addiction. The Ventral Pallidum (VP) is a key structure in the basal forebrain that is important for motivation and reward processing and prior work has shown that VP neuronal populations encode the expected outcome value. However, the sensitivity of these representations to a change in the expected outcome value remains poorly understood. Outcome (reward) devaluation, in which the value of the expected outcome is diminished, is a classic test of goal-directed behavior that allows for testing whether the neural representations of expected outcome value change with a change in the value of the expected outcome. In this study, we used in-vivo electrophysiology to record from single units in the VP of adult rats responding to Pavlovian reward cues before and after reward devaluation. Adult rats (n= 5, 3 males, 2 females) were trained with an auditory conditioned stimulus (CS+) that signalled delivery of a 10% sucrose reward and a control cue (CS-) that never predicted reward. We then recorded from the VP of these rats during training and reward devaluation sessions. Reward devaluation was achieved via sensory specific satiety by allowing the animals free access to either sucrose (thereby altering its expected value) or a control substance (maltodextrin). As expected, we found that many VP neurons (65%) were excited by the CS+, and that these neurons were more responsive to the CS+ than the CS-. Additionally, VP responses to the CS+ were reduced after free consumption of sucrose. Interestingly, free access to maltodextrin also reduced VP responses to the CS+, suggesting that these changes in VP responding were not due to the encoding of the sucrose reward specifically. Future experiments will aim to investigate whether VP neurons respond more selectively to other forms of devaluation. Overall, this study will enhance our understanding of expected outcome value encoding within the VP and how that representation changes with a change in the value of the expected outcome.

Disclosures: A. Sood: None. **J.M. Richard:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.03

Topic: G.03. Motivation

Support: SRP, Occidental College

Title: Retrograde tracing reveals forebrain afferents to the anterior or posterior ventral pallidum in the rat brain

Authors: *R. G. KOLPA[1], K. R. URSTADT[1], K. C. BERRIDGE[2];
[1]Cognitive Sci., Occidental Col., Los Angeles, CA; [2]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The Ventral Pallidum (VP) is a brain structure involved in limbic functions including disgust, hedonia, feeding, and motivation. However, inputs to this region are not well established. Using retrograde tract tracing, we identified potential forebrain afferents to the VP. Tracer solutions of 2% Fluorogold or 0.25% cholera toxin subunit B (CTB) were micro-iontophoretically injected into various locations within or near the anterior or posterior VP, and traced neurons were labeled with tyramide signal amplified immunofluorescence. Labeled neurons were then quantified in various forebrain regions. Anterior VP injections primarily labeled large numbers of neurons in the Accumbens Core, Accumbens Shell, and Caudate Putamen. Meanwhile, posterior VP injections labeled moderate numbers of neurons in the Lateral Septum, Magnocellular Preoptic nucleus, Paraventricular Thalamus, Basomedial Amygdala, insular cortex, nucleus of the horizontal limb of the diagonal band, piriform cortex, and olfactory tubercle. Posterior VP injections also labeled small numbers of neurons in the bed nucleus of the stria terminalis, medial preoptic area, sublenticular extended amygdala, lateral hypothalamus, and paraventricular hypothalamus. Most regions demonstrating labeling are associated with regulation of pleasure, feeding, and/or reward processing, according to past literature. This suggests that these regions may regulate these functions, in part, via the VP.

Disclosures: R.G. Kolpa: None. **K.R. Urstadt:** None. **K.C. Berridge:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.04

Topic: G.03. Motivation

Support: NIH 1R01DA044199
NSF IOS1557987

Title: Interrupting VP activity during acquisition of cue relationships and sign-tracking behavior

Authors: *E. SMEDLEY, K. S. SMITH;
Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Appetitive Pavlovian autoshaping, where cues precede a reward delivery, can produce several conditioned responses in animals. Some animals produce a sign-tracking response whereby they come to interact with the cue and the cue attains incentive value beyond its predictive properties (i.e., the cue itself becomes wanted). Previous studies have demonstrated that the ventral pallidum (VP) plays a critical role in the sign-tracking behaviors. Notedly, many autoshaping procedures used single lever cues to predict reward and previous work has described how cues learned in sequence will both gain a sign-tracking response, with a bias toward the first lever in sequence, and are each able to later update and inform the other cue and impact subsequent sign-tracking behavior (Smedley & Smith, 2018a, 2018b). Given the VPs role in sign-tracking, and how neurons in the VP represent the incentive value of sign-tracked cues, we suspected that the VP will be involved in representing many cues in sequence. Further, it would likely have the capability of building associations between serially sign-tracked cues and modifying these relationships if outcomes change (i.e., the cue mediated extinction effect seen when one cue in sequence is extinguished). Here, rats were implanted with bilateral optical fibers, and transduced with either a virus containing archaerhodopsin (eArch3.0) or a control virus (eYFP) in the VP. Preliminary results indicate that inhibition of VP during distal lever delivery does not alter the quantity of sign-tracking behavior. However, animals with VP inhibition during the distal cue were faster to subsequently interact with the proximal lever. Notedly, the differences in latency to respond did not persist into subsequent testing days after lever extinction indicating that this effect may only be present in acquisition. These findings suggest that VP may be modulating the timing and vigor of the sign-tracked behavioral response.

Disclosures: E. Smedley: None. **K.S. Smith:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.05

Topic: G.03. Motivation

Support: National Institute of Mental Health R01MH110594
National Institute of Mental Health R01MH116937
McKnight Foundation award

Title: Neuronal mechanisms of novelty seeking

Authors: *T. OGASAWARA[1], F. SOGUKPINAR[3], K. ZHANG[4], Y.-Y. FENG[4], J. PAI[1], A. JEZZINI[1], I. E. MONOSOV[1,3,4,5,2];
[1]Neurosci., [2]Pain Ctr., Washington Univ. Sch. of Med., Saint Louis, MO; [3]Electrical Engin., [4]Biomed. Engin., [5]Neurosurg. Sch. of Med., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Humans and other primates interact with the world by observing and exploring visual objects. In particular, they often seek out the opportunities to view novel objects that they have never seen before, even when they have no extrinsic primary reward value. However, despite the importance of novel visual objects in our daily life, we currently lack an understanding of how primate brain circuits control the motivation to seek out novelty. We found that novelty-seeking is regulated by a small understudied subcortical region, the zona incerta (ZI). In a task in which monkeys made eye movements to familiar objects to obtain the opportunity to view novel objects, many ZI neurons were preferentially activated by predictions of future novel objects and displayed burst excitations before gaze shifts to gain access to novel objects. Low intensity electrical stimulation of ZI facilitated gaze shifts, while inactivations of ZI reduced novelty-seeking. Surprisingly, additional experiments showed that this ZI-dependent novelty-seeking behavior is not regulated by canonical neural circuitry for reward prediction. The habenula-dopamine pathway, known to reflect reward predictions, was relatively inactive during novelty-seeking behavior in which novelty had no extrinsic reward value. Instead, high channel-count electrophysiological experiments and anatomical tracing identified a prominent source of control signals for novelty-seeking in the anterior ventral medial temporal cortex (AVMTC), a brain region known to be crucially involved in visual processing and object memory. In addition to their well-known function in signaling the novelty or familiarity of objects in the current environment, AVMTC neurons reflected the predictions of future novel objects, akin to the way neurons in reward-circuitry predict future rewards in order to control reward-seeking. Our data uncover a network of primate brain areas that regulate novelty-seeking. The behavioral and neural distinctions between novelty-seeking and reward-prediction highlight how the brain can accomplish behavioral flexibility, providing a mechanism to explore novel objects.

Disclosures: T. Ogasawara: None. **F. Sogukpinar:** None. **K. Zhang:** None. **Y. Feng:** None. **J. Pai:** None. **A. Jezzini:** None. **I.E. Monosov:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.06

Topic: G.03. Motivation

Support: NIMH R01MH123544

Title: Importance of CRF+ neurons in the anterior bed nucleus of the stria terminalis for avoidance, reward, and motivation behaviors

Authors: *I. MAITA[1], P. HU[2], K. WIERSIELIS[3], T. A. ROEPKE[4], B. A. SAMUELS[5];
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Abstract: The bed nucleus of the stria terminalis (BNST) plays a valence surveillance role, integrating inputs and regulating Hypothalamo-Pituitary Adrenal responses. BNST is highly heterogenous, sexually dimorphic, and composed of diverse nuclei and cell populations. One subregion, the anterior BNST (aBNST), is populated by GABAergic Corticotropin-Releasing Factor (CRF)- expressing neurons, which are important regulators of avoidance behaviors. However, CRF+ neurons in BNST also project to brain areas involved in reward and motivation behaviors. Here we used both optogenetic and chemogenetic approaches to dissect the importance of aBNST CRF+ neurons in avoidance, reward, and motivation behaviors in both male and female C57BL/6J mice.

Disclosures: I. Maita: None. **P. Hu:** None. **K. Wiersielis:** None. **T.A. Roepke:** None. **B.A. Samuels:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.07

Topic: G.03. Motivation

Title: Oxytocin decreases expression of sucrose conditioned place preference via mechanisms in the ventral tegmental area

Authors: *M. SUNDAR[1], K.-C. LEONG[2];
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Abstract: Understanding the neural processes underlying natural reward-seeking behavior, for rewards such as sugar, has widespread implications. While the reward circuit serves an important

adaptive function for physiological and psychological health, maladaptation of this circuit may be attributed to various disorders including addiction, eating disorders, diabetes, and obesity. Recently, the neuropeptide oxytocin (OXT) has been shown to modulate reward-related behaviors and processes. The present study examined how OXT affects sucrose-seeking behaviors in rodents using the conditioned place preference (CPP) paradigm, which serves to measure reward seeking and the expression of context-reward associations. In Experiment 1, Sprague-Dawley male rats underwent conditioning trials in which they were given sucrose pellets (45 mg) in a distinctive environment or no reward in a separate environment. On test day, rats were injected peripherally with OXT (1 mg/kg; i.p.; n = 7) or saline (n = 7) prior to the CPP test. The systemic administration of OXT attenuated the expression of sucrose CPP. The ventral tegmental area (VTA) is a key structure in the reward circuit, responsible for mediating various reward-related processes for natural and drug rewards, and also shows robust expression of OXT receptors. In Experiment 2, rats underwent the same behavioral paradigm as in Experiment 1. On test day rats received an intra-VTA infusion of OXT (0.5 µg/0.5 µL; n = 6) or saline (0.5 µL; n = 7). Intra-VTA OXT administration resulted in attenuation of sucrose CPP expression, suggesting that the modulatory effect of OXT is mediated by processes within the VTA. These results provide valuable information about oxytocin's mechanisms for reducing addictive behaviors and encourage its potential as a therapeutic for addiction.

Disclosures: M. Sundar: None. **K. Leong:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.08

Topic: G.03. Motivation

Support: NIH Grant 1R01DA044199
NSF Grant IOS1557987

Title: Cholinergic transmission in the nucleus accumbens core alters the flexibility of sign-tracking responses

Authors: *E. S. TOWNSEND, K. A. AMAYA, E. B. SMEDLEY, K. S. SMITH; Dartmouth Col., Hanover, NH

Abstract: Reward predictive cues can acquire their own motivational value. The physical manifestation of this motivation is often cue-directed Pavlovian conditioned approach, commonly referred to as sign-tracking. In dynamic environments, sign-tracking behaviors must be flexible enough to change as well, or the animal will risk losing out on rewarding outcomes. Thus, after the introduction of an omission schedule, where deflection of a lever-cue cancels

delivery of the reward, animals flexibly express sign-tracking by morphing their behavior to align with the new cue information (e.g., increases in non-contact behaviors such as sniffing and orienting, and overall avoidance of lever deflection) without changes in cue attraction. However, neural mechanisms underlying this type of flexibility remain unclear. One area of interest is the nucleus accumbens core (NAcC). While its role in governing sign-tracking behavior itself is well known, the modulatory role of a small population of cholinergic interneurons in the NAcC has received little attention. Recently, these cells have been studied with respect to other forms of motivation. Still, it is unknown what role cholinergic transmission plays in the regulation of sign-tracking behavior during the omission condition. Using pharmacological manipulations in rats that actively displayed sign-tracking behavior, nicotinic receptors in the NAcC were inhibited by administering mecamylamine, a nicotinic antagonist, during the first five days of the omission schedule following sign-tracking acquisition. Reduction of nicotinic activity in the NAcC suppressed the rats' ability to flexibly alter their sign-tracking responses during omission in contrast to controls, although nicotinic inhibition did not entirely prevent the rats from eventually compensating for the omission schedule. Behavioral microanalysis suggested that rats with inhibited nicotinic receptor signaling engaged in more food port directed behaviors and displayed delayed changes in cue-directed, non-contact behaviors such as sniffing and orienting relative to controls. Altogether, these results indicate that activity at NAcC nicotinic receptors are important for animals to integrate information into their sign-tracking behavior in a way that aids motivational flexibility.

Disclosures: E.S. Townsend: None. **K.A. Amaya:** None. **E.B. Smedley:** None. **K.S. Smith:** None.

Poster

P676. Motivation: Subcortical Neurocircuitry III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P676.09

Topic: G.03. Motivation

Support: Productos Medix 3247
Fundación Miguel Alemán
Catedra Marcos Moshisky (to R.G.)

Title: The complementary GABAergic and Glutamatergic neuron activity in the lateral hypothalamus orchestrates palatability-guided consumption.

Authors: *M. LUNA[1], A. COSS[2], O. X. GUERRERO GUTIERREZ[1], A. GARCIA[3], R. GUTIERREZ[1];
[1]Pharmacol., [2]Electrical Engin., CINVESTAV, Ciudad de Mexico, Mexico; [3]Neuroscience, Cell. Physiol. Inst., UNAM, Ciudad de Mexico, Mexico

Abstract: Obesity has become a major global health concern. One culprit of this epidemic is the overconsumption of sucrose, which due to its high palatability (hedonic value) promotes its intake beyond homeostatic need. The Lateral Hypothalamic Area (LHA) coordinates fundamental behaviors, including sleep-wake cycles, feeding, motivation, stress, and nociception. Optogenetic and chemogenetic approaches have revealed that the GABAergic (LHA^{GABA}) and glutamatergic (LHA^{Glut}) populations participate in consummatory and avoidance behavior, respectively. In our previous work, extracellular recordings on mice revealed that LHA contains a subpopulation of palatability-related neurons; these were optotagged as GABAergic and non-GABAergic (perhaps glutamatergic), and they responded directly and inversely proportional to sucrose's palatability, respectively. Thus, we hypothesized that complementary activation of LHA^{GABA} and silencing of LHA^{Glut} neurons could encode sucrose's palatability. To this aim, we used transgenic mice (Vgat-ires-Cre and Vglut2-ires-Cre) and induced expression GCaMP6m on LHA^{GABA} and LHA^{Glut} neurons. We employed fiber photometry and a Brief Access Taste Test (BATT), giving 4s access to either an empty sipper, water, 3%, or 18% sucrose. The lick rate was used as a descriptor of palatability. We found that LHA^{GABA} neuron activity was directly proportional to the palatability of the tastants, while LHA^{Glut} neuronal activity was inversely related. It is plausible that this antithetical modulatory pattern between LHA^{GABA} and LHA^{Glut} neurons allows palatability-guided consumption. We used $LHA^{Vgat\text{-}ChR2}$, $LHA^{Vgat\text{-}GtACR2}$, $LHA^{Vglut2\text{-}ChR2}$, and $LHA^{Vglut2\text{-}GtACR2}$ mice to test the causal effect of activating (with ChR2) or silencing (with GtACR2) these neurons with real-time place preference and closed-loop two bottle conditioning. We found that activating LHA^{GABA} or silencing LHA^{Glut} neurons made a less preferred side or sucrose bottle more preferred. In contrast, silencing LHA^{GABA} or activating LHA^{Glut} neurons resulted in avoiding the more preferred photostimulated side or bottle. Finally, open-loop experiments with solid foods (Chow, High-Fat Diet (HFD), and sucrose pellet) confirmed that well-fed $LHA^{Vgat\text{-}ChR2}$ and $LHA^{Vglut\text{-}GtACR2}$ mice increased interaction time and consumption of the most palatable food available. Besides, in 20 h fasted $LHA^{Vgat\text{-}GtACR2}$ and $LHA^{Vglut2\text{-}ChR2}$ mice avoided HFD and only consumed it in the unstimulated period. Our findings shed light on the functional difference of these two subpopulations of neurons and how they might complement each other to induce rewarding and aversive behaviors.

Disclosures: M. Luna: None. **A. Coss:** None. **O.X. Guerrero Gutierrez:** None. **A. Garcia:** None. **R. Gutierrez:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.01

Topic: G.03. Motivation

Support: NIH R01MH102456

Title: Distinct involvement of hypothalamic oxytocin cell populations in juvenile social play behavior

Authors: *S. M. BOWDEN[1], A. M. LUXHOJ[1], A. R. TATONE[1], V. GRINEVICH[2], R. BREDEWOLD[1], A. H. VEENEMA[1];
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Abstract: Juvenile social play is a highly motivated and rewarding behavior displayed across a variety of mammalian species, including rats and humans. Deficits in social play are a hallmark symptom in children diagnosed Autism Spectrum Disorder (ASD). Although the oxytocin (OXT) system has been implicated as a potential therapeutic in ASD, little is known about its role in juvenile social play. Here, we determined the involvement of OXT cell populations in the paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus (SON) in the modulation of social play behavior in juvenile rats. To achieve this, an excitatory DREADD construct under the control of the OXT promoter was infused into either the PVN or SON of 26-day-old rats. Social play behavior was assessed one week later over two trials, in which either clozapine-*N*-oxide or saline was administered 30-minutes prior to testing in a randomly assigned counterbalanced manner. In a first round of experiments in females, we found that chemogenetic activation of PVN^{OXT} cells selectively increased the duration of social play, while chemogenetic activation of SON^{OXT} cells selectively increased social investigation. Current studies are underway to determine the effects of chemogenetic activation of PVN^{OXT} and SON^{OXT} cells on social play behavior in males. Because ASD disproportionally affects males, we hypothesize to find sex differences in the role of hypothalamic OXT in social play behavior. Together, these experiments demonstrate a differential involvement of PVN^{OXT} cells and SON^{OXT} cells in the modulation of social behavior in juvenile female rats. Future studies will explore the neural circuitry by which PVN^{OXT} cells modulate social play behavior in both sexes. Overall, this research aims to provide insights into the potential sex-specific use of OXT-based therapeutics to alleviate social play deficits in children with ASD.

Disclosures: S.M. Bowden: None. **A.M. Luxhoj:** None. **A.R. Tatone:** None. **V. Grinevich:** None. **R. Bredewold:** None. **A.H. Veenema:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.02

Topic: G.03. Motivation

Support: NSF IOS 1735934
NIH R01 MH102456
NSF DBI 1906523

Title: Sex-specific activation of oxytocin receptor expressing cells within nodes of the social behavior neural network following social investigation

Authors: *K. E. YOEST[1], A. R. BARRETT[2], M. H. HENRY[1], H. A. VELISEK[1], D. ANDERSON[4], A. H. VEENEMA[3];
[1]Psychology, [2]Neurosci., [3]Dept. of Psychology, Michigan State Univ., East Lansing, MI; [4]Univ. Ana G. Mendez, San Juan, PR

Abstract: The social behavior neural network (SBNN) is an interconnected circuit of brain regions that aid in the regulation of a wide variety of social behaviors. Within this network, the neuropeptide oxytocin (OXT) and its corresponding receptor (Oxtr) play a prominent role in which sex differences in the organization of the OXT system have been linked to the sex-specific regulation of social behaviors in adult rats. Sex differences in the OXT system in some nodes of the SBNN are already present early in development, but the functional implications at a young age remain unknown. Here, we determined how activation of Oxtr expressing cells within three nodes of the SBNN differs in juvenile male and female rats following exposure to a social stimulus. Using in situ hybridization, we measured the number of cells expressing Oxtr mRNA as well as fos mRNA (as a marker of neuronal activation) within the bed nucleus of the stria terminalis (BNST), ventromedial hypothalamus (VMH), and medial amygdala (MeA). In the BNST of males, but not females, exposure to a social stimulus increased the proportion of fos positive cells that expressed Oxtr without altering the overall number of cells that were activated, indicating a shift in the population activity of the BNST. Conversely, in the VMH, females have greater expression of both fos positive cells and Oxtr expressing cells that are fos positive than males, suggesting higher overall activity in the VMH of females. Ongoing work seeks to determine whether patterns of Oxtr cell activation within the MeA resemble those seen in the BNST or in the VMH. Together, these results indicate that specific nodes of the SBNN show differential recruitment of Oxtr cells in response to social experience in juvenile rats and provide insight into how OXT may act across SBNN nodes to coordinate age and sex-specific responses to social stimuli.

Disclosures: K.E. Yoest: None. **A.R. Barrett:** None. **M.H. Henry:** None. **H.A. Velisek:** None. **D. Anderson:** None. **A.H. Veenema:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.03

Topic: G.03. Motivation

Support: R01MH102456
IOS1735934

DGE1848730

Title: Structural sex differences in the ventral pallidal vasopressin system are associated with the sex-specific regulation of social play behavior in juvenile rats

Authors: *J. D. A. LEE, C. J. REPPUCCI, E. D. M. HUEZ, S. M. BOWDEN, R. BREDEWOLD, A. H. VEENEMA;
Michigan State Univ., East Lansing, MI

Abstract: Social play is displayed by juveniles of many mammalian species, including rats and humans, and engagement in social play helps develop social competence throughout life. Children diagnosed with autism spectrum disorder, a sex-biased neurodevelopmental disorder, show decreased involvement in social play, thus emphasizing the need to understand the neural mechanisms underlying social play in both sexes. We recently showed that vasopressin (AVP) acting in the lateral septum regulates social play behavior in juvenile rats and AVP acting in the ventral pallidum (VP) regulates sociosexual motivation in adult rats, with AVP modulating both behaviors in a sex-specific manner. However, whether intra-VP AVP signaling is involved in the sex-specific regulation of juvenile social behaviors, such as social play, is unknown. In this study, we first examined the structure of the intra-VP AVP system in juvenile rats and found multiple sex differences, with denser AVP-immunoreactive fibers and vasopressin 1a receptor (V1aR) binding in males compared to females, but a greater number of v1aR+ cells in females compared to males. However, when we examined AVP inputs to the VP originating from the bed nucleus of the stria terminalis (BNST), we found no sex differences in the number of avp+ cells in the BNST or in the proportion of VP-projecting BNST avp+ cells. We are currently determining whether there are sex differences in the recruitment of BNST avp+ cells following exposure to social play. In addition, we are examining whether there are sex differences in AVP inputs to the VP originating from the posterodorsal medial amygdala (MePD) and in the recruitment of MePD avp+ cells following exposure to social play. Next, in order to determine the functional role of intra-VP AVP signaling in regulating social play behavior, we investigated whether exposure to social play altered recruitment of VP v1aR+ cells and determined the causal involvement of intra-VP AVP signaling in the regulation of social play by infusing a specific V1aR antagonist into the VP prior to social play exposure. We found that exposure to social play enhanced recruitment of VP v1aR+ cells in males only. However, V1aR blockade in the VP increased social play duration in males but decreased social play duration in females compared to same sex control groups. Together, these findings demonstrate robust structural sex differences in the VP-AVP system that may underlie the sex-specific regulation of social play behavior by the VP-AVP system. Furthermore, these findings provide insights into the involvement of AVP signaling across a network of brain regions in the sex-specific regulation of social play behavior in juvenile rats.

Disclosures: J.D.A. Lee: None. **C.J. Reppucci:** None. **E.D.M. Huez:** None. **S.M. Bowden:** None. **R. Bredewold:** None. **A.H. Veenema:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.04

Topic: G.03. Motivation

Support: MH119422

Title: 5HT2C receptors mediate age- and stress-dependent social interactions

Authors: *A. J. NG, B. H. BRADY, L. K. VINCELETTE, J. P. CHRISTIANSON; Psychology and Neurosci., Boston Col., Chestnut Hill, MA

Abstract: Social interaction allows for the transfer of affective states among community members in a phenomenon referred to as “emotional contagion.” The behaviors and expressions of individuals experiencing pain and fear can evoke anxiety-like states in observers which shape subsequent social interactions. The serotonin 2C receptor (5HT2C) is an important central regulator of anxiety, and we hypothesized that social reactions to stressed individuals may be mediated by 5HT2C receptors systemically and in the insular cortex, an area involved in the approach/avoidance decisions with stressed others. We administered the selective 5HT2C antagonist SB242084 both systemically (10mg/kg*)* and directly into the insula (5μM*)* and found that both treatments prevented the approach and avoidance, respectively, of stressed juvenile (PN30) or stressed adult (PN50) conspecifics in the social affective preference test. Interestingly, direct infusion of the selective 5HT2C agonist CP809101 (5μM) was insufficient to alter social interaction with juvenile or adult conspecifics. On a multielectrode array, bath application of CP809101 augmented glutamatergic synaptic transmission as evidenced by increased field excitatory postsynaptic potentials (fEPSP) peak amplitude in what appears to be a dose dependent manner (data collection is ongoing). We conclude that observation of a stressed other evokes serotonin release and binding at insular 5HT2C receptors which may underlie an empathetic anxiety-like state and shape social decision making.

Disclosures: A.J. Ng: None. **B.H. Brady:** None. **L.K. Vincelette:** None. **J.P. Christianson:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.05

Topic: G.03. Motivation

Support: NIH Grant MH119422

Title: The role of the basolateral amygdala and its projections to the insula in social investigation of stressed adult rats

Authors: *A. DJERDJAJ[1], A. J. NG[1], N. WORLEY[1], B. CAREY[1], J. P. CHRISTIANSON[2]; [2]Psychology, [1]Boston Col., Chestnut Hill, MA

Abstract: Social vocalizations, chemosignals, and behavioral expressions of one's internal affective state shape the selection of social behavior responses made by others who detect these cues. A network of interconnected brain structures called the social decision making network (SDMN) integrates these stimuli and network output yields social behaviors that are appropriate for a given interaction. Deficits in SDMN connectivity underlie several psychiatric disorders such as schizophrenia and autism so further understanding the function of the SDMN may grant us new insight into aberrant social behaviors. The basolateral amygdala (BLA) and the insular cortex (IC) are reciprocally connected and each contribute to social and emotional behaviors. The BLA is commonly viewed as a valence encoder while the IC mediates approach and avoidance behaviors. Prior work from our lab showed this BLA-IC tract is necessary for social investigation of stressed juveniles (PN30) in a social affective preference (SAP) paradigm in which a test rat is presented with 2 conspecifics, 1 naïve to treatment and 1 stressed via 2 footshocks. Here, we explored the involvement of these BLA projections to the IC in a SAP test with 2 adult conspecifics (PN50). Test rats prefer interaction with the naive adult in this paradigm. Local inhibition of the BLA was achieved via bilateral transduction of AAV-hSyn-hM4D(Gi)-mCherry followed by systemic administration of clozapine-N-oxide (3mg/kg) prior to SAP tests with adult conspecifics. Further, tract-specific inhibition of IC-projecting BLA neurons was achieved via bilateral transduction of AAV-hSyn-hM4D(Gi)-mCherry in the BLA, bilateral cannulation in the IC, and CNO injection (1μM) directly into the IC prior to SAP testing with adult conspecifics. Ongoing investigations include transsynaptic tracing to identify BLA-IC projection patterns and manipulate these specific IC neurons receiving monosynaptic input from the BLA. Additionally, in vivo Ca2+ imaging of both BLA neurons and BLA-IC neurons aims to describe neuronal activity of these general and projection-defined neurons during encounters with naive and stressed conspecifics. The current results expand on previous studies investigating the role of this tract in social affective preference for juvenile conspecifics. Taken together, these studies identify a new tract by which social emotional information is processed within the SDMN.

Disclosures: A. Djerdjaj: None. **A.J. Ng:** None. **N. Worley:** None. **B. Carey:** None. **J.P. Christianson:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.06

Topic: G.03. Motivation

Support: University of Wisconsin Start-up Fund 135-135G848
NIH R01 MH119041

Title: Neural circuit mechanisms underlying the medial preoptic area-mediated social play behavior in rats

Authors: *C. ZHAO, L. RITERS;
Integrative Biol., Univ. of Wisconsin-Madison, Madison, WI

Abstract: The medial preoptic area (MPOA) is well-known for its role in sexual and maternal reward, but it is also implicated in pair-bond maintenance behavior and centrally involved in non-sexual, affiliative song and reward in birds. Recent studies from our laboratory demonstrate that MPOA is a central nucleus in which opioids act to govern non-sexual affiliative, highly rewarding social play behavior in adolescent rats. However, the neural circuit mechanisms underlying the MPOA-mediated social play behavior remain largely unresolved. The MPOA contains a large cluster of neuronal populations that send direct efferent projections to approximately 20 brain areas. The MPOA to ventral tegmental area (VTA) pathway is involved in motivation and reward associated with sexual and maternal behaviors. We hypothesize that this projection pathway may also mediate social play behavior through a positive and rewarding mechanism. Additionally, the MPOA projects to the periaqueductal gray (PAG), which is well-known for its role in pain and aversive emotional states such as fear and anxiety. Affiliative social interactions are not only rewarding but also reduce negative affective states. We hypothesize that the signal pathway from the MPOA to PAG may promote non-sexual affiliative interactions (i.e., social play) via reducing a negative affective state. To begin to test these hypotheses, we combined retrograde tract tracing with immediate early gene expression and immunofluorescent labeling for mu opioid receptors (MORs) to identify opioid-sensitive projection pathways from the MPOA to VTA and PAG that are activated after performance of social play. Retrograde tracer, Fluoro-Gold (FG), was microinjected into the VTA or PAG. Immediate early gene expression (i.e., Egr1) was assessed and triple immunofluorescent labeling of MOR, Egr1 and FG in the MPOA was performed after social play. We revealed that play animals displayed an increase in neurons double-labeled with Egr1 and FG (i.e., activated projection neurons), and triple-labeled with MOR, Egr1 and FG (i.e., activated MOR projection neurons) in the MPOA when compared to no-play rats. The increased activation of projection neurons that express MORs from MPOA to VTA or PAG after social play suggests that opioids may act through these projection pathways to govern social play. Collectively, this study provides neuroanatomical evidence supporting that projection pathways from the MPOA to VTA and PAG are involved in the modulation of social play by MORs in the MPOA.

Disclosures: C. Zhao: None. **L. Riters:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.07

Topic: G.03. Motivation

Support: NIH 1R01DA044199
NSF IOS1557987

Title: Exploring sex-based differences in sign-tracking compulsivity following outcome devaluation

Authors: *E. A. BIEN[1], K. S. SMITH[2];
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Abstract: Forming habits to get rewards can be helpful for efficient behavior; however, if those behaviors become compulsive when change is needed, they can be unhealthy. Sign-tracking, in which animals motivationally engage with a reward cue, has proven to be a useful readout for kinds of reward-seeking that can be either inflexible or flexible depending on different conditions. It needs to be determined if sign-tracking behavior itself shows sex differences that match the sex differences that we see in human disorders characterized by maladaptive reward seeking. To determine this, we allowed male and female rats (outbred Long Evans; Charles River) to first develop a sign-tracking response to a reward-predictive cue. Then, we used a conditioned taste aversion-mediated outcome devaluation (OD) procedure to assess how much animals continued to sign-track for a devalued reward. First, we found, surprisingly, that males and females did not differ in sign-tracking levels during acquisition and did not differ in the speed with which they acquired sign-tracking behavior. We also found that female and male rats exposed to LiCl decreased their pellet consumption during the OD task and decreased their sign-tracking behavior after the task compared to their female and male saline control counterparts; again, however, sex was not found to influence these decreases in behavior. Males and females exposed to LiCl showed similar decreases in pellet consumption and decreases in sign-tracking behavior after OD. These results were unexpected given that previous studies in Sprague Dawley rats found that female rats have more stimulus-directed Pavlovian Approach than males and that females are more insensitive to OD (e.g., Hammerslag and Gulley, 2014; Pitchers et al., 2015). In conclusion, our findings with this type of rat strain show that sex does not affect sign-tracking behavior, devaluation of its outcome, or how sign-tracking adjusts to the devalued outcome. This suggests that genetic or other unknown factors can contribute to whether sex plays any role in variations in cue-directed motivation.

Disclosures: E.A. Bien: None. **K.S. Smith:** None.

Poster

P688. Motivation: Social Communication and Behavior in Rats

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P688.08

Topic: G.09. Drugs of Abuse and Addiction

Title: The effect of pair-housing on ethanol conditioned place preference in male rats

Authors: *E. LORENZ, H. BALLAS, K.-C. LEONG;
Trinity Univ., San Antonio, TX

Abstract: According to the 2019 National Survey on Drug Use and Health (NSDUH), 14.5 million people above the age of twelve reported suffering from alcohol use disorder (AUD). While social support has been shown to maintain sobriety in individuals, social support may also protect against the development of the addiction. The objective of this study was to understand the relationship between social interaction and alcohol reward processes in male Sprague Dawley rats using an alcohol conditioned place preference model in combination with pair-housing. Pair-housed and single-housed rats received conditioning trials in which ethanol exposure (20% v/v 1.0 mg/kg; orally administered) was paired with a distinctive environment while a separate environment was paired with saline exposure (orally administered). Our results revealed that pair-housed rats displayed diminished alcohol conditioned place preference relative to single-housed rats, as indicated by their time spent in the alcohol-paired context. The neuropeptide oxytocin has previously been established in both drug reward and social interaction, however, the effects of social interaction on drug reward is not fully understood. We also examined the role of oxytocin on mediating the effect of pair-housing on alcohol conditioned place preference through co-administration of an oxytocin receptor antagonist. These results investigate the role of oxytocin in driving the attenuating effect of pair-housing on alcohol reward-related behaviors.

Disclosures: E. Lorenz: None. **H. Ballas:** None. **K. Leong:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.01

Topic: G.09. Drugs of Abuse and Addiction

Support: F32 AA027935
R01 MH48404

Title: Adolescent voluntary alcohol drinking impairs response inhibition in adulthood and alters cortical-striatal dynamics in male and female rats

Authors: *A. MCCANE[1], B. MOGHADDAM[2];
[2]Behavioral Neurosci., [1]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Development of alcohol use disorder (AUD) is strongly associated with initiation of drinking during adolescence. A better understanding of the impact of alcohol exposure on the adolescent brain in the context of motivated behavior is fundamental for understanding the etiology and pathophysiology of AUD. Deficits in response inhibition are a critical feature of AUD and are a clinically relevant endophenotype of the disorder. However, little is known about the functional consequences of adolescent alcohol drinking on response inhibition and related cognitive measures in adulthood. Here, we used a moderate voluntary adolescent drinking model combined with a recently developed Cued Response Inhibition Task (CRIT) that assesses response inhibition, stimulus-response relationships, attentional processes and behavioral flexibility in adolescents or adults. We find adult rats who drank ethanol during adolescence made more (irrelevant) nose pokes during the inter-trial-interval, were quicker to make premature responses, and exhibited reduced response inhibition, relative to sucrose exposed controls. We are currently investigating the mechanistic basis of this observation by recording from single units and local field potential activity in two brain regions implicated in response inhibition, the orbitofrontal cortex (OFC) and dorsal striatum (DS), with power, OFC-DS synchrony, firing rate and spike correlations being computed during all behavioral epochs. Our preliminary analysis of recording data reveals ethanol exposed rats show an enhanced response to reward in the OFC and an augmented response in the DS during premature responding. Because dysfunction of cortico-striatal circuits is a critical feature of AUD, these translational result will enhance our mechanistic understanding of brain changes that occur in these circuits as a consequence of adolescent ethanol exposure.

Disclosures: A. McCane: None. **B. Moghaddam:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.02

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA024109

Title: Anterior insular cortex activity encodes aversion-resistant alcohol consumption

Authors: *P. STARSKI[1], M. MORNINGSTAR[2], S. KATNER[4], R. SANDLER[4], S. WEAN[4], C. C. LAPISH[3], F. W. HOPF[5];
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Abstract: Compulsive alcohol drinking is a core facet of alcohol use disorder, where alcohol intake persists despite adverse consequences, and a major clinical obstacle to successful treatment. Recent work has shed light on underlying circuit and neurochemical mechanisms in aversion-resistant drinking, but still remain largely undefined. Previously, we have described that the anterior insular cortex, a critical mediator of emotion, motivation, and interoception, plays a significant role in promoting aversion-resistant drinking. Indeed, optogenetic inhibition of the insula-ventral striatum network significantly decreased compulsive alcohol drinking in rat as observed when the aversive stimulus was a bitter-tasting quinine or intermittent footshock. However, these same inhibitions had no effect during alcohol-only drinking, suggesting this insula-related circuit is critical specifically for aversion-resistant drinking. Additionally, heavy human drinkers show similar insula-circuit recruitment when responding for alcohol under threat of shock. Together, these studies indicate that the insula plays a significant role, in both rodent and human, when overcoming conflict (compulsion) to drink alcohol. Here, we hypothesized that alcohol intake paired with aversive challenge will increase firing levels in the anterior insula described by 32-electrode multi-wire electrophysiology implants. Rats were first trained to consume 20% alcohol through an intermittent intake paradigm over 3 months. Quinine, a bitter additive, was given at two concentrations (10 mg/L and 60 mg/L) to assess aversion-resistant, compulsive motivation for alcohol drinking. Animals demonstrating compulsive drinking were implanted with multi-wire recording probes, allowing insula neuron activity to be recorded during alcohol-only and two levels of aversion-resistant drinking. Licking patterns were assessed concurrently with lickometry. Similar to previous findings, rats maintain longer licking bouts during alcohol-only sessions, with shorter bouts during quinine-alcohol drinking. Insular electrophysiological recording has revealed firing data of over 1200 neurons from 13 rats during alcohol-only and moderate (10 mg/L) and high (60 mg/L) quinine challenge in alcohol. Ongoing analyses focus on the comparison of anterior insular neuron firing characteristics between drinking conditions with one main hypothesis being greater insula firing scaling to the level of challenge and amount of consumption.

Disclosures: P. Starski: None. **M. Morningstar:** None. **S. Katner:** None. **R. Sandler:** None. **S. Wean:** None. **C.C. Lapish:** None. **F.W. Hopf:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.03

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 AA014445-16
P50 AA026117-04
T32 AA007565-28

Title: Chronic ethanol and withdrawal dysregulates glutamatergic function in 'reward' and 'aversion' basolateral amygdala neurons in a circuit- and sex-dependent manner

Authors: M. PRICE, *B. MCCOOL;
Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The basolateral amygdala (BLA) regulates aversion and reward processing during alcohol withdrawal through projections to the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAC), respectively. Glutamatergic pyramidal neurons in the BLA receive glutamatergic projections from two distinct input pathways. Midline structures like the medial prefrontal cortex send projections through the *stria terminalis* (ST) whereas lateral cortical areas send projections through the external capsule (EC). Previous studies revealed that chronic intermittent ethanol and withdrawal (CIE/WD) increases glutamate release from ST inputs and increases postsynaptic function at EC-BLA synapses. In this study, we compared neurophysiological changes in CIE/WD-exposed 'reward' and 'aversion' BLA neurons to understand the neurophysiological mechanisms involved in anxiety and alcohol intake in male and female rats. We used retrograde labeling of BLA neurons projecting to the NAC (BLA-NAC) or BNST (BLA-BNST) as representative 'reward' and 'aversion' BLA neurons, respectively. A 2-way ANOVA revealed that CIE/WD increased glutamate release from ST inputs onto BLA-BNST neurons in male and female rats (main effect of CIE/WD $p<0.001$). There were no significant effects in BLA-NAC neurons. In contrast, increased postsynaptic function at EC-BLA synapses is both circuit- and sex-dependent. A 2-way ANOVA showed that CIE/WD increased postsynaptic function at EC synapses with BLA-NAC neurons, independent of sex (main effect of CIE/WD $p<0.01$). In BLA-BNST neurons, there was a main effect of CIE/WD ($p<0.01$) and an interaction effect ($p<0.05$), and post-hoc tests revealed that CIE/WD increased postsynaptic function at EC synapses with BLA-BNST neurons only in females ($p<0.01$). These findings indicate that CIE/WD dysregulates the neurophysiology of 'reward' and 'aversion' BLA neurons through distinct mechanisms, and that female rats are more vulnerable to changes in 'aversion' BLA neurons than males. We then investigated the effects of increased glutamatergic function on BLA neuron excitability in male rats. Using mixed effects analyses, we found that CIE/WD increased the excitability of BLA-NAC (main effect of current $p<0.0001$, interaction effect $p<0.05$) and BLA-BNST neurons (main effect of current $p<0.0001$, interaction effect $p<0.0001$) when only GABAergic transmission was blocked. However, the enhanced excitability was abolished in BLA-NAC and BLA-BNST neurons when both glutamatergic and GABAergic transmission were blocked, supporting the idea that increased glutamatergic function facilitates BLA excitability after CIE/WD.

Disclosures: M. Price: None. **B. McCool:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.04

Topic: G.09. Drugs of Abuse and Addiction

Support: F31 AA028722
R01 AA014445
R01 AA023999
P50 AA026117

Title: Chronic ethanol exposure potentiates nucleus basalis magnocellularis (NBM) neurotransmission and facilitates pathological release of glutamate in the BLA during ethanol withdrawal

Authors: *S. SIZER, B. MCCOOL;
Wake Forest Univ., Winston Salem, NC

Abstract: Chronic ethanol exposure potentiates basolateral amygdala (BLA) glutamatergic afferents, driving BLA pyramidal neuron hyperexcitability and the expression of anxiety during withdrawal (WD). The purpose of the present study was to understand the contribution of nucleus basalis magnocellularis (NBM) cholinergic projections to withdrawal-induced alterations in glutamate neurophysiology in the BLA. Following 10 days of chronic intermittent ethanol (CIE) vapor exposure and 24 hours of withdrawal, we completed *ex vivo* whole-cell patch clamp electrophysiology and utilized optogenetics to bidirectionally manipulate NBM terminal activity before recording electrically-evoked paired-pulse ratios (PPRs) as a measure of presynaptic function. Specifically, we compared presynaptic nicotinic acetylcholine receptor (nAChR)-dependent changes in stria terminalis (ST) glutamate PPR between CIE animals and age-matched AIR controls. Optical excitation of NBM terminals significantly increases ST glutamate release probability in AIR animals (one-way repeated-measures ANOVA, $p<0.01$), with no significant effect in CIE animals. Mecamylamine (MEC; non-selective nAChR antagonist) perfusion reverses this effect, illustrating that laser stimulation of NBM cholinergic projections activates presynaptic nAChRs on ST glutamate terminals. In contrast, optical inhibition of NBM terminals attenuates withdrawal-induced increases in ST glutamate release probability in CIE neurons (mixed effects ANOVA, $p<0.05$). In AIR neurons, laser inhibition of NBM terminals show no appreciable effects on ST glutamate PPR (mixed effects ANOVA, $p>0.05$). Laser activation of NBM terminals alone elicits a monosynaptic EPSC that is potentiated following CIE exposure and withdrawal. Together, these data strongly indicate that CIE/WD strengthens NBM neurotransmission and produces tonic activity of nAChRs on ST glutamatergic synapses, resulting in a ‘pathological’ elevation of glutamate release. These findings illustrate that CIE-dependent modifications to NBM neurophysiology may profoundly shift the excitatory/inhibitory balance in the BLA.

Disclosures: S. Sizer: None. **B. McCool:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01-AA027544
NIDA T32-DA007234
NIDA 1P30-DA048742-01A1
University of Minnesota Doctoral Dissertation Fellowship

Title: Ethanol-induced suppression of GIRK-dependent signaling in the basal amygdala

Authors: *E. MARRON[1], M. E. TIPPS[1], B. HAIDER[2], T. R. ROSE[2], B. N. VO[2], M. C. DEBAKER[3], K. D. WICKMAN[1];
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Abstract: Repeated cycles of ethanol intoxication and withdrawal are thought to induce neuroadaptations in brain regions that regulate mood, goal-directed behavior, and learning, promoting key features of alcohol use disorder (AUD). The GABAB receptor (GABABR) is a critical regulator of the behavioral sensitivity to drugs of abuse and has emerged as a promising therapeutic target for AUD treatment. Our work and others has shown that psychostimulants can suppress GABABR-dependent signaling in key neuron populations in the mesocorticolimbic system, including the ventral tegmental area and the prelimbic cortex. Despite the established involvement of limbic structures in AUD, the potential influence of drug exposure on GABABR-dependent signaling in these brain regions remains unknown. Here, we examined the impact of alcohol exposure on GABABR-dependent signaling in the basolateral amygdala (BLA). Using two distinct ethanol exposure approaches, we found that the somatodendritic inhibitory effect of GABABR activation on principal neurons in the basal (BA) sub-region of the BLA (p=0.0274, n=12-13/group), but not the lateral (LA) (p=0.7144, n=8-10/group), was reduced during withdrawal from ethanol. Experiments using KO animals and pharmacological manipulations showed that this adaptation was mediated by a selective weakening of the G protein-gated inwardly rectifying K+(GIRK/Kir3) channel component of the GABABR-induced response. Similar to other brain regions studied, this GABABR-GIRK response in the BA is driven by heterotetramers of GIRK channels containing both GIRK1 and GIRK2 subunits. Interestingly, although the GIRK3 subunits do not seem to participate in channel formation, the neuroadaptation was absent in GIRK3KO animals (p=0.7529, n=11/group), suggesting a modulatory role of this subunit. We next sought to identify a behavior correlate of this

neuroadaptation by performing a battery of behaviors. Ethanol withdrawal produced robust physical dependence indicated by a handling induced convulsions test (p=0.0001, n=12/group), anxiety-like phenotype in the light/dark box test (p=0.0041,n=12/group), anxiety/compulsive-like phenotype in the marble burying test (male: p=0.0296, n= 6/group; female: p=0.0453, n=6/group) and increased irritability-like behavior in the bottle brush test (male: p=0.0007, n=6/group; female: p=0.0002, n= 6/group). Interestingly, preliminary data obtained from constitutive GIRK3KO animals show GIRK3 subunit ablation prevents these behavioral changes. These results suggest that the behavioral effects induced by ethanol exposure withdrawal might be, at least in part, mediated by GIRK3.

Disclosures: E. Marron: None. **M.E. Tipps:** None. **B. Haider:** None. **T.R. Rose:** None. **B.N. Vo:** None. **M.C. DeBaker:** None. **K.D. Wickman:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.06

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT IA202120

Title: Neuroadaptive structural changes of an intermittent access to alcohol model in wistar rat.

Authors: *D. ANGELES-VALDEZ[1], A. LOPEZ-CASTRO[2], S. ALCAUTER[2], L. NARANJO[3], E. GARZA-VILLARREAL[2];
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Abstract: Alcohol use disorder (AUD) is a complex neuropsychiatric disorder that combines behavioral, neurobiological, and psychosocial alterations. The development of AUD has been characterized as a three-stage cycle: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. Although these stages have been studied in humans and animals, there is still missing knowledge of the neuroadaptative changes happening across the brain during each stage. Knowledge of these changes would possibly help us understand how AUD is established. Preclinical models of AUD are key to model these stages, and in combination with longitudinal in vivo neuroimaging, could provide a better understanding of the neuroadaptative changes in AUD. In this study, we used the Intermittent-Access Ethanol 2-Bottle-Choice Drinking Paradigm in n= 29 (16 female), Wistar rats, starting at a weight of 160 g (∼7weeks old−45 days post-natal corresponding to adolescence). Animals had ad-libitum access to standard

laboratory chow and water. After the baseline MRI scans, 22 rats were assigned to the EtOH group and 7 to the Control group. EtOH group underwent a modified version of the Wise pre-clinical AUD model where, briefly, rats received continuous access to 20% ethanol on a 12-hour reversed light/dark cycle for 45 days. The first acquisition was at baseline, and the second MRI acquisition was after finishing the 20 sessions (45 days, P90) of ethanol intake. We used deformation-based morphometry to create Jacobian maps per subject and time point using a Two Level DBM tool using a Fisher rat atlas template and parcellation, with mixed model GLM, we compared the within-subjects effect of baseline versus 20 sessions of intermittent alcohol. The main alcohol intake (g/Kg/24 h) was 1.90 +-2.08 (mean/ +-sd). For the group x session interaction. We found local volume changes after 20 sessions of voluntary alcohol consumption in rats, thalamus, cerebral cortex showed reduction while Dentate Gyrus and Caudate-Putamen showed an increase. In order to find the brain morphological phenotype associated with alcohol consumption, two classes were created (high consumption / low consumption). Finally, a Bayesian logistic regression model was used to determine the brain morphology associated with alcohol consumption. These changes may arguably be neuroadaptive structural changes related to chronic alcohol use, however, he results are not corrected for multiple comparisons.

Disclosures: D. Angeles-Valdez: None. **A. Lopez-Castro:** None. **S. Alcauter:** None. **L. Naranjo:** None. **E. Garza-Villarreal:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex, age and beta-endorphin expression interact to alter the effect of alcohol on open field behavior in mice

Authors: *K. LEONARD[1], H. JONES[1], M. WALDRON[2], J. E. GRISEL[2], *E. RHINEHART[1]; [1]Susquehanna Univ., Selinsgrove, PA; [2]Bucknell Univ., Lewisburg, PA

Abstract: Alcohol causes enduring changes in the motivation and reward neurocircuitry, and Alcohol Use Disorder (AUD) affected 19.3 million Americans aged 12+ in 2018. One common denominator of rewarding, potentially addictive substances, including alcohol, is an acute increase in central mesolimbocortical dopaminergic pathway activity concomitant with psychomotor stimulation in behavioral tests, such as the open field test (OFT). Previous research has shown that sex, age, and expression of the opioid peptide, beta-endorphin (BE), affect the behavioral responses to alcohol in tests such as the elevated plus maze and the light-dark box. Therefore, we hypothesized that OFT behavior after a single acute alcohol administration would be dependent upon age, sex and BE expression. To test this hypothesis, we examined the impact

of EtOH treatment (1.5g/kg, n=62 or 1.75g/kg, n=61) on OFT behavior in adult (n=62) and adolescent (n=61), male (n=58) and female (n=65) C57BL/6J mice that either expressed BE (BE +/+, n=53) or lacked BE (BE-/-, n=70). As expected, sex, genotype and EtOH dosage all interacted to influence the amount of time animals spent in the center part of the open field arena ($p<0.05$), an indicator of anxiety-like behaviors in rodents. In addition, age, sex and genotype all independently impacted distance traveled in the center of the arena ($p<0.05$), an indicator of psychomotor stimulation. For example, at 1.75 g/kg EtOH, the adolescent, female, BE+/+ animals traveled a significantly greater distance while in the center of the arena than their male counterparts (12.6 ± 2.5 vs. 18.8 ± 2.1, $p<0.05$), unlike the adolescent, BE -/- animals that showed no sex difference (8.0 ± 2.0 for males vs. 6.2 ± 1.5 for females, n.s.). These data indicate that it is necessary to simultaneously consider a complex array of factors to determine how any one individual experiences the subjective anxiolytic and stimulant effects of alcohol. At the end of the behavioral study animals were sacrificed and brains were perfused with paraformaldehyde and sectioned at 35 microns to be processed immunohistochemically for both tyrosine hydroxylase, a marker for dopaminergic neurons, and c-fos, a protein marker of neuronal activation to allow us to determine if differences in the activation of dopaminergic neurons correlate with any of the observed differences OFT behavior (data in progress). These data will help us better understand how several physiological factors interact to determine the initial subjective experience of alcohol and how that relates to site specific neuronal activation, which may shed light on the mechanisms responsible for differences in individual susceptibility to AUD.

Disclosures: K. Leonard: None. **H. Jones:** None. **M. Waldron:** None. **J.E. Grisel:** None. **E. Rhinehart:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.08

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of physical activity and alcohol ingestion on female mouse brain GABA-B receptor concentrations

Authors: *T. J. BUHR[1], C. H. REED[2], E. E. BAUER[1], A. SHOEMAN[1], R. J. VALENTINE[2], P. J. CLARK[1];
[1]Food Sci. and Human Nutr., Iowa State Neurosci. Interdepartmental Grad. Program, Ames, IA; [2]Kinesiology, Iowa State Univ., Ames, IA

Abstract: Alcohol misuse (e.g. binge-drinking) is a risk factor to alcohol dependence and a major contributor to the development of numerous health problems. Evidence suggests that

regularly misusing alcohol can result in adaptations in the metabotropic GABA-B receptor system, which remains a popular pharmacological target to treat alcohol dependence. Moreover, evidence suggests that exercise can cause alterations in brain GABA systems, including GABA-B receptor mediated signaling. Therefore, we investigated the possible interactions between alcohol misuse and chronic mild physical activity on GABA-B receptor protein levels across the brain, as it may have important implications for the treatment of alcohol use disorders. Adult female C57/BL6J mice received 2.5hr access to voluntary running wheels daily or remained sedentary for 35 days. During the last five days following wheel access, the voluntary binge-like drinking paradigm, "drinking in the dark" (DID), was employed, whereby runner and sedentary mice received access to either a 20% ethanol solution or water for a 2-4 hour period in their home cages approximately 3 hours into the dark cycle (i.e. active period). Mice were sampled immediately following DID and the prefrontal cortex, hypothalamus, striatum, cerebellum, hippocampus, brainstem, and remaining cortical areas were rapidly microdissected. GABA-B receptor protein density across brain regions was assessed via western blot. 35 days of moderate physical activity had no effect on amounts of ethanol ingested during DID. Preliminary data indicate that GABA-B receptor 1 levels increase with exercise, but were unaffected by alcohol, in the prefrontal cortex. Possible exercise-increased GABA-B receptor protein densities may hold significance in treatment of not only alcohol use disorders, but also treatment of other substance abuse and stress-related mental health disorders.

Disclosures: T.J. Buhr: None. **C.H. Reed:** None. **E.E. Bauer:** None. **A. Shoeman:** None. **R.J. Valentine:** None. **P.J. Clark:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant AA027978

Title: A behavioral approach for sex-specific increase in ethanol consumption after repeated stress in mice

Authors: *V. GARCIA-RIVAS, M. THOMAS, Y. S. MINEUR, M. R. PICCIOTTO;
Dept of Psychiatry, Yale Univ., New Haven, CT

Abstract: Recent epidemiological data suggest that, compared to men, women are more likely to drink alcohol to cope with stress and negative affect. This stress-induced drinking is problematic, as it may put women at higher risk for developing alcohol use disorders. Even though the precise neurobiological mechanisms behind this phenomenon remain unknown, there is converging

evidence suggesting that exposure to chronic ethanol dysregulates the neural networks in the basolateral amygdala, which may prime for exacerbated stress responses in a sex-dependent manner, which can be then alleviated by acute ethanol intake. To test this hypothesis, we first developed a behavioral model that captures this female-specific increase in ethanol drinking after repeated stress. Adult male (n=10) and female (n=10) C57BL6J mice were trained to drink ethanol daily in a modified “drinking-in-the-dark” (DID) paradigm, which consisted of introducing two sippers (water and 10% ethanol diluted in water), to their home cage for 2 hours, 3 hours into their dark phase. Once drinking behavior was stabilized after 12 daily sessions, mice were split into control (n=5 per sex) and *"stress"* (n=5 per sex) groups for the remainder of the experiment. On days 13, 14, 20 and 21, *"stress"* mice underwent stress sessions at least 4 hours prior to the corresponding DID for that day. These stress sessions consisted of 1hr exposure to mild foot shocks, applied at semi-random intervals in specialized chambers equipped with an electrified grid. During this time, control mice remained in their home cage undisturbed. An increase in alcohol intake in the *"stress"* female group was observed on the last stress day (day 21), compared to control female mice and male subgroups. Differences in alcohol seeking between female *"stress"* and control mice was also apparent when the same mice were then later trained to seek for an oral 10% ethanol reward using an FR3 operant alcohol self-administration paradigm. This behavioral approach is well suited to be combined with fiber photometry or optogenetics in freely moving mice, to assess how sex-specific neural dynamics in the basolateral amygdala at the intersection between chronic alcohol and repeated stress may put females at risk of maladaptive ethanol drinking.

Disclosures: V. Garcia-Rivas: None. **M. Thomas:** None. **Y.S. Mineur:** None. **M.R. Picciotto:** None.

Poster

P741. Alcohol: Circuitry and Neurophysiology of Addiction IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P741.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIHR01AA024109

Title: Sex dependent behavioral strategies for compulsion-like alcohol drinking

Authors: *T. DE OLIVEIRA SERGIO[1,2], J. KELLNER[1], D. DAREVSKY[2], V. RACHETTI[3], A. F. JONES[1], F. W. HOPF[1,2];
[1]Indiana Univ., Indianapolis, IN; [2]Univ. of California San Francisco, San francisco, CA; [3]Univ. Federal of Rio Grande do Norte, Natal, Brazil

Abstract: Sex dependent behavioral strategies for compulsion-like alcohol drinking. Thatiane de Oliveira Sergio[1,2], Jacob Kellner[1], David Darevsky[2], Vanessa De Paula Soares Rachetti[3], Andrea F Jones[2], Frederic W Hopf[1,2].[1]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN.[2]Department of Neurology, University of California, San Francisco, San Francisco, CA[3] University Federal of Rio Grande do Norte, Natal, BrazilCompulsive alcohol drinking, where intake persists despite adverse consequences, is a central driver of excessive alcohol drinking and major obstacle to treating alcohol use disorder. Hazardous drinking in women has dramatically increased across the last decades, making it critical to understand sex-specific mechanisms driving pathological intake and develop more targeted anti-addiction therapies. Using lickometry, we examined microstructural behavioral changes that can reflect altered motivation, as well as different behavioral strategies, between males and females during conflict-resistant intake of quinine-alcohol (QuiA). Overall, it was widely observed that females drank more under all conditions. Quinine-sensitivity in three different concentrations (10, 60 and 100mg/L) was similar across sexes, however, females showed differences in the bout length for the highest concentrations of quinine. Together, our findings indicate that females and males have different strategies for maintaining alcohol drinking in the face of negative consequences. Knowing these differences can be useful to better understand the drinking problem in females.Supported by R01AA024109.

Disclosures: T. De Oliveira Sergio: None. **J. Kellner:** None. **D. Darevsky:** None. **V. Rachetti:** None. **A.F. Jones:** None. **F.W. Hopf:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.01

Topic: H.03. Decision Making

Support: NIMH grant R01MH057414

Title: Emotional modulation of memory: a computational model shows how excessive amygdala input to the hippocampus can lead to overgeneralization of fear-related memories

Authors: *Y. J. JOHN[1], J. WANG[3], D. BULLOCK[2], H. BARBAS[1];
[1]Neural Systems Lab, Dept of Hlth. Sci., [2]Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA; [3]Dept. of Psychological and Brain Sci., Univ. of California at Santa Barbara, San Diego, CA

Abstract: The emotional system of the brain has powerful effects on processing of context and episodic memory. A key pathway in the circuit mediating these effects is the projection from the amygdala to the anterior hippocampus (ventral in rodents). Wang and Barbas (2018) uncovered a

distinctive pattern in the amygdalar projections to hippocampal neurons in primates. The projections from amygdala to the CA3 subfield targeted excitatory pyramidal neurons as well as parvalbumin (PV) and calretinin (CR) inhibitory neurons. Evidence suggests that the inhibitory PV and CR interneurons act on CA3 pyramidal neurons in a manner analogous to their counterparts in cortex: PV provides strong perisomatic inhibition of pyramidal (excitatory) neurons, whereas CR provides disinhibition by inhibiting dendrite-targeting inhibitory interneurons. Amygdalar projections to the CA1 subfield were found to differ from those to CA3 by not targeting PV interneurons. The projections primarily targeted pyramidal neurons and CR interneurons, indicating synergistic excitatory and disinhibitory effects. Given that CA1 is the primary source of hippocampal outputs, understanding the overall effect of the amygdala on hippocampal function requires modeling the combined effects of its inputs to the integrated CA3-CA1 circuit. We therefore developed a computational model of spiking neurons that demonstrates some key functional implications of these projections. Our simulations show that the amygdala-CA3 projection can serve as a potent mechanism for two emotion-guided processes: (1) gating of CA3-dependent mnemonic and contextual processes, and (2) reduction of episodic richness in CA3. A moderate level of amygdala excitation can boost CA3 activity broadly, enabling rich detail to be available for discrimination and associative learning. But abnormally high amygdala activity can suppress “non-emotional” inputs to CA3 from other sources, leading to loss of detail. Our simulations also show that amygdala input to CA1 modulates the transition between a phasic “pattern-match” mode and a tonic “pattern-mismatch” mode vital for new learning. These effects of the amygdala on the anterior hippocampus suggest a mechanistic basis for “sticky” overgeneralization in cases of elevated fear, and, more broadly, for anxiety-related shifts in the processing of context and episodic memory.
Supported by NIH grants (NIMH).

Disclosures: Y.J. John: None. **J. Wang:** None. **D. Bullock:** None. **H. Barbas:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.02

Topic: H.03. Decision Making

Support: NIH Grant R01MH117785

Title: Density of parvalbumin, calbindin, and calretinin inhibitory neurons in subgenual cingulate area 25 of the neurotypical human

Authors: M. RAKOCZY, ***M. JOYCE**, B. ZIKOPOULOS, H. BARBAS;
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Abstract: Brodmann Area 25 (A25) in the posterior ventromedial prefrontal cortex is linked with areas associated with learning, memory, motivation, and reward. Hyperactivity in A25 has been observed in humans with depression. In marmosets, pharmacological overactivation increases anxiety- and anhedonia-like behaviors. Inhibition is a critical component of neuronal circuitry, and disruptions in the equilibrium between inhibition and excitation can lead to pathological changes in brain circuits. The hyperactivity observed in A25 in depression may involve an imbalance between the activity of excitatory and inhibitory neurons. To investigate the inhibitory neuron composition of the neurotypical subgenual cingulate in *post-mortem* human brains, we used immunohistochemistry to label the calcium-binding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR), which label almost all cortical inhibitory neurons and have different functional roles in local inhibition. Here we used stereology, a method of unbiased systematic random volumetric sampling, to estimate the laminar density of PV, CB, and CR neurons in A25 of *post-mortem* neurotypical human brains for comparison with previous data in the rhesus macaque. The primate A25 contains CB positive pyramidal neurons that stain lightly and can be distinguished from darkly labeled CB positive inhibitory neurons. In this study, only the darkly labeled CB neurons were included. We found that CR neurons are denser in A25 than PV and CB. CR neurons are concentrated most densely in superficial layers I-IIIa of A25. Conversely, PV neurons are predominantly found in mid-deep layers III-VI, while CB dark-labeled neurons have a more even distribution among layers, with slightly higher density in mid-superficial layers II and IIIa. These findings are comparable to recently published data in the rhesus macaque. CR and CB neurons in superficial layers are thought to fulfill a disinhibitory or modulatory role, while PV neurons exert a strong inhibitory effect by targeting pyramidal neurons perisomatically. These data provide the basis for future comparison with data from brains with psychiatric disease states, including depression.

Disclosures: M. Rakoczy: None. **M. Joyce:** None. **B. Zikopoulos:** None. **H. Barbas:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.03

Topic: H.03. Decision Making

Support: NIH Grant R01MH118500

Title: Impaired Sleep Spindles in Schizophrenia: Studying Underlying Mechanisms with a Biophysical Neural Model

Authors: N. MATUK, B. ZIKOPOULOS, *A. YAZDANBAKHSH;
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Abstract: Sleep spindles are brief oscillations present during EEG recordings that can be seen during nonrapid eye movement sleep and are implicated in memory consolidation. Spindles originate within the thalamocortical loop, which consists of the inhibitory thalamic reticular nucleus (TRN), thalamocortical relay, and cortical neurons. The thalamocortical circuity is further subdivided into a matrix and core, the former is implicated in limbic processing and memory consolidation, while the latter is associated with sensory processing. Although the mechanism responsible for generating spindles is understood, their functionality is still largely unknown. Therefore, the full extent of the functional consequences of spindle impairments in schizophrenia are not well understood. In this study, we built a detailed circuit-based, biophysical neural model of the thalamocortical loop, incorporating Boltzmann equations for voltage and neurotransmitter-gated channels, both ionotropic and metabotropic, to address our key question of why sleep spindle frequency and density decrease in schizophrenia. We tested a variety of connectivity changes to replicate patterns of spindles observed in schizophrenia as well as neurotypical individuals. By comparing the reversed engineered circuit that generates schizophrenia-like spindles with functional imaging and electrophysiological data we were able to create a prioritized list of changes within the circuit to further investigate as potential mechanisms responsible for the spindle deficits in schizophrenia. With models of isolated matrix and core thalamocortical loops, our studies focused on reduced TRN inhibition and disruption of reciprocal thalamocortical connectivity, both at the level of the thalamus and the cortex, as potential mechanisms. Incorporating changes in cortical feedback in order to mimic cortical “up” and “down” states was also investigated to understand spindling and bursting in different states. Our model acts as a tool to direct future imaging and electrophysiology experiments to identify the neural mechanisms underlying schizophrenia spindle impairments.

Disclosures: N. Matuk: None. **B. Zikopoulos:** None. **A. Yazdanbakhsh:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.04

Topic: H.03. Decision Making

Support: NIH Grant R01 MH118500

Title: Molecular and synaptic features in thalamic neural circuits of primates

Authors: X. LIU, J. SON, L. TRAVIS, N. ZAMAN, K. SKUBISZ, K. WASIF, S. KONSTANTINIDOU, E. ANGELOPOULOS, J. NGO, H. BARBAS, ***B. ZIKOPOULOS**; Boston Univ., Boston, MA

Abstract: The primate thalamus forms two parallel and distinct thalamocortical (TC) circuits with reciprocal components: the core and matrix, which drive and modulate TC activity. The core TC circuit, prevalent in sensory thalamus, drives activity focally in the middle cortical layers and gets feedback through small modulatory cortical axon terminals from pyramidal neurons in layer 6 (L6). The matrix TC circuit can drive activity in high-order thalamus, through large axon terminals from cortical layer 5 (L5) pyramidal neurons and sends broad thalamic feedback to the superficial cortical layers. The inhibitory thalamic reticular nucleus (TRN) intercepts all TC communication and is situated strategically between the thalamus and cortex. However, little is known about the molecular and synaptic profiles of core and matrix thalamic circuits and their interactions in primates. To begin to address this gap, we used immunostaining, brightfield, fluorescent, confocal, and electron microscopy to systematically examine and quantitatively map key molecular and synaptic features, including markers of excitatory, inhibitory, and calcium-binding proteins in a primarily core thalamic nucleus, the visual lateral geniculate nucleus (LGN), a high-order nucleus, the mediodorsal (MD), and the TRN. We found predominantly core neurons in LGN, while the expression of core and matrix markers was more mixed in MD with more matrix neurons in the medial, limbic MD. The LGN had significantly fewer inhibitory neurons compared to MD, with the lateral, parvicellular MD, containing the highest density of GABAergic interneurons. We also found that core and matrix thalamocortical tracts passing through TRN were organized following a dorsal-ventral gradient, with relatively more matrix tracts dorsally and more core tracts ventrally. Further examination of synaptic profiles in thalamic nuclei at high resolution, showed a majority of excitatory synapses, mainly from the cortex, with variable shapes and sizes, in all nuclei. Moreover, a significant proportion of large inhibitory synapses targeted the soma and proximal segments of TC projection neurons in LGN, MD, and inhibitory TRN neurons. Our results provide support for the anatomical and functional organization of core and matrix TC circuits and highlight the prominent role of local and external inhibition in the primate thalamus, which provides additional flexibility to TC processing.

Disclosures: X. Liu: None. **J. Son:** None. **L. Travis:** None. **N. Zaman:** None. **K. Skubisz:** None. **K. Wasif:** None. **S. Konstantinidou:** None. **E. Angelopoulos:** None. **J. Ngo:** None. **H. Barbas:** None. **B. Zikopoulos:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.05

Topic: H.03. Decision Making

Support: NIH Grant RO1MH057414

Title: Pathways linking the Amygdala, Hippocampus, and Subgenual Area 25 in the Primate Nucleus Reuniens

Authors: *L. G. MARSHALL[1], M. P. JOYCE[1], S. BANIK[1], J. WANG[1], J. HOLZ[1], D. XIAO[1], J. G. BUNCE[2], H. BARBAS[1];
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Abstract: The primate thalamic Reuniens Nucleus (RE) is the most ventral of the limbic anterior midline nuclei, situated directly above the third ventricle. The RE is distinguished among thalamic nuclei by its bidirectional connections with the hippocampus and medial prefrontal cortex (mPFC). In rats, detailed investigation has demonstrated RE's role in several cognitive functions that require hippocampus-mPFC activity coordination, including spatial working memory, memory consolidation, and strategy shifting. However, in the primate, several foundational questions remain unanswered: What is the chemoarchitecture of RE? Are other limbic structures, such as the amygdala, also connected with RE? To address the chemoarchitecture of the RE, we used immunohistochemistry to label GABA, a marker of inhibitory neurons, and for the calcium-biding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR), all of which label excitatory projection neurons in the thalamus. PV neurons are associated with a core thalamocortical connectional system that terminates in middle layers of cortex. CB and CR are associated with a matrix profile of thalamocortical connections, with terminations in the upper layers 1-3a and layer 6. We found that the RE is primarily populated by CB and CR neurons with sparse PV neurons and processes, classifying it as a matrix-dominant thalamic nucleus. Injections of neural tracers in subgenual cingulate cortical area 25, amygdala, and hippocampus showed robust incoming and outgoing connections with the RE in all three pathways. The hippocampal and posterior area 25 pathways were densest in anterior RE, while the amygdala had a consistent density of connections throughout the anterior to posterior axis of RE. We also used high resolution methods to examine ultrastructural characteristics of incoming connections from the three pathways that form synapses in RE. We observed multiple instances of amygdala axonal boutons forming successive synapses with the same postsynaptic structure. We also observed some hippocampal terminations forming multisynaptic complexes that are comparable to synaptic triads formed by amygdala terminations in the thalamic Mediodorsal Nucleus. In summary, the primate RE shows a specialization by its strong connection with the amygdala. The connections of RE with the hippocampus, area 25, and amygdala positions it to coordinate activity among these regions and is likely an integral node in limbic memory and emotional networks.

Disclosures: L.G. Marshall: None. **M.P. Joyce:** None. **S. Banik:** None. **J. Wang:** None. **J. Holz:** None. **D. Xiao:** None. **J.G. Bunce:** None. **H. Barbas:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.06

Topic: H.03. Decision Making

Support: Wellcome Trust (206330/Z/17/Z)

Title: Role of hippocampus in reward valuation under uncertainty and for effort

Authors: B. ATTAALLAH, P. PETITET, R. ZAMBELLAS, S. IRANI, S. MANOHAR, *M. HUSAIN;
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Abstract: The hippocampus plays an important role in many functions including memory and spatial cognition. Converging evidence from animal and human studies indicates that it might also play an important role in motivation and value-based decision making. This contribution, however, is under-investigated and yet to be established.
Here, we assessed people with focal hippocampal damage (N=19) as a result of autoimmune limbic encephalitis. We investigated how they make value-based decisions in two contexts using two behavioural paradigms. In the first task, participants made decisions in the context of uncertainty. In a passive form of this paradigm, they decided simply whether to accept or reject potential rewards in exchange for tolerating uncertainty. In an active search form, they could gather information, which came at a cost, to reduce uncertainty prior to committing to their decisions. In the second paradigm, participants made decisions in the context of effort, accepting/rejecting decisions based on whether the reward on offer was worth the effort assigned to it.
The results showed that people with hippocampal damage, in comparison to healthy controls, have reduced sensitivity to changes in reward when evaluated against uncertainty, but not effort. While maintaining intact sensitivity to uncertainty, the influence of changes in reward (in the passive task) and information cost (in the active task) was blunted in hippocampal patients. Moreover, this reduced sensitivity to changes in reward was more pronounced in patients with more severe hippocampal atrophy. In contrast, performance of hippocampal patients on the effort task was intact.
These results suggest a context-dependent role of the hippocampus in reward processing and valuation, which might represent an important step forward in understanding hippocampal contributions to value-based decision making and motivated behaviour in general.

Disclosures: B. Attaallah: None. **P. Petitet:** None. **R. Zambellas:** None. **S. Irani:** None. **S. Manohar:** None. **M. Husain:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.07

Topic: H.03. Decision Making

Support: IRP NIMH/NIH

Title: Visualizing neurons in the reward circuit based on their co-projections

Authors: *A. LUZ-RICCA, W. LERCHNER, J. SCHEIDT, K. E. DASH, M. A. ELDRIDGE, B. J. RICHMOND;
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Abstract: The orbital frontal cortex (OFC) and rostromedial caudate (rmCD) are brain regions considered important for reward value processing and decision-making. The OFC is thought to be involved mainly in reward value assessment, whereas the rmCD is thought to play a role in reward expectation and action selection. To identify neurons that project from other areas to both of these regions, we injected retrograde viruses expressing red and green fluorescent reporters into the OFC and rmCD, respectively. After injection of the viral vectors, histological analysis showed a population of neurons in many regions co-expressing both fluorescent reporters, indicating that these neurons co-project to the OFC and rmCD. These neurons were located within higher order visual areas (TE and V4) but were also identified in brain areas involved with processing spatial and emotional salience, such as the amygdala, rhinal cortex, and insula. Perhaps surprisingly, in the hemisphere contralateral to the injection site, these same regions had a larger than expected percentage of co-projecting neurons relative to neurons projecting to either OFC or rmCD alone. For example, in the amygdala of the ipsilateral hemisphere, approximately 20% of the neurons projecting to OFC also projected to rmCD, but from the contralateral amygdala nearly all OFC-projecting neurons also projected to rmCD. We are further characterizing the population of co-projecting neurons, which presumably carries identical signals to two brain regions thought to underlie reward evaluation and may play a role in the normalization of sensory information.

Disclosures: A. Luz-Ricca: None. **W. Lerchner:** None. **J. Scheidt:** None. **K.E. Dash:** None. **M.A. Eldridge:** None. **B.J. Richmond:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.08

Topic: H.03. Decision Making

Support: NIH/NIMH/IRP

Title: A role for projection neurons from monkey's rhinal cortex to rostromedial caudate in stimulus-reward associations

Authors: *W. WANG[1], M. A. ELDRIDGE[1], N. MIYAZAKI[1], J. E. PEARL[1], W. LERCHNER[1], T. SETOGAWA[2], J. N. TURCHI[1], B. LI[1], B. J. RICHMOND[1];
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Abstract: Primates, including old-world monkeys, learn stimulus-reward associations seemingly without effort. We previously showed that rhinal cortex has a causal role in associating value with visual stimuli, with monkeys without a rhinal cortex being less sensitive to previously learned stimulus-reward associations and learning new associations between visual cues and reward schedule states slowly, if at all. Rorstromedial caudate (rmCD) also plays a causal role in stimulus-reward associations. When rmCD is inactivated chemogenetically, monkeys show a deficit in evaluating the size of the upcoming reward signaled by visual cues. There is a known connection between deep layers of the rhinal cortex projecting to rmCD. Here, we interrupted this connection by combining a unilateral rhinal cortex lesion with contralateral expression of an hM_4Di DREADD via a retrograde lentivirus (FugE) injected into rmCD. The monkey was trained to perform a task with four visual cues, which signaled the upcoming reward of one, two, four or eight drops of water, respectively. A new set of cues was used each day. Without chemogenetic inactivation, the monkey learned quickly to accept only the two larger sizes of reward and refuse the smaller rewards. However, when all neurons projecting to rmCD were silenced by systemically injected DCZ, the monkey needed significantly more trials to learn the stimulus-reward mapping. The monkey's decision time also increased. To manipulate projection neurons from rhinal cortex to rmCD selectively, on some days, microinjections (3ul x 4 sites, [100nM]) of DCZ were made into perirhinal cortex. The microinjections led to a small but significant deficit in the stimulus-reward mapping. Thus, our results show that projection neurons from rhinal cortex to rmCD are causally involved in the learning of visual stimulus-reward associations.

Disclosures: W. Wang: None. **M.A. Eldridge:** None. **N. Miyazaki:** None. **J.E. Pearl:** None. **W. Lerchner:** None. **T. Setogawa:** None. **J.N. Turchi:** None. **B. Li:** None. **B.J. Richmond:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.09

Topic: H.03. Decision Making

Support: IRP NIMH/NIH

Title: A possible role for divisive normalization in the primate value-reward circuit

Authors: *W. LERCHNER[1], J. SCHEIDT[3], A. LUZ-RICCA[3], K. E. DASH[2], M. A. ELDRIDGE[4], B. J. RICHMOND[5];
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Abstract: Signals for reward value are distributed widely throughout the brain and are thought to be important for evaluating sensory stimuli when faced with behavioral choices. One mechanism for normalizing the value of sensory information is divisive normalization. In the early visual system normalization of luminance is achieved through feedback inhibition of multifurcating complex cells onto simple cells. At the other end of the decision-making hierarchy, there is evidence for divisive normalization in human and old-world monkey choice behaviors. Here we present evidence for the existence of a widespread neuron population that could be part of a substrate for normalizing the value of sensory signals by co-projecting to the orbital prefrontal cortex (OFC) and the rostromedial caudate (rmCD) from both hemispheres. OFC and rmCD are considered key structures for evaluating and updating reward signals. We identified this co-projecting neuron population in two monkeys by unilaterally injecting a retrograde lentivirus expressing a fluorescent protein with one color into OFC and a retrograde lentivirus expressing another color into rmCD. Clusters of individual neurons that expressed both colors, and thus projected to both regions, were found throughout the temporal lobe and other cortical regions responsible for processing visual and emotionally relevant information, including insular cortex and the dorsomedial thalamus. The highest percentages of co-projecting neurons relative to neurons only projecting to one of the two regions were found in areas of the basal and medial amygdala rich in dopamine innervation, the parahippocampus (TF) and the temporal parietal occipital area (TPO). In the same regions in the contralateral hemisphere, co-projecting neurons were found in smaller numbers but with a much higher percentage of co-projecting neurons compared to populations only projecting to the OFC or rmCD individually. Any signal responsible for normalization of value-reward association would need to be collected from sensory processing regions of both hemispheres, making this neuron population a prime candidate for sending identical value information to OFC and rmCD, which could be used to feed back a signal for adjusting gains in sensory processing areas.

Disclosures: W. Lerchner: None. **J. Scheidt:** None. **A. Luz-Ricca:** None. **K.E. Dash:** None. **M.A. Eldridge:** None. **B.J. Richmond:** None.

Poster

P781. Corticolimbic Circuits in Learning, Memory and Reward Valuation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P781.10

Topic: H.03. Decision Making

Support: NSERC Discovery Grant RGPIN-2020-05577
FRQS Junior 1 Salary Award #284309
BBRF Young Investigator Grant #27298

Title: Irrational choice via curvilinear value geometry in ventromedial prefrontal neurons

Authors: K. JUREWICZ[1], B. J. SLEEZER[2], P. MEHTA[2], B. Y. HAYDEN[3], ***B. EBITZ**[1]; [1]Univ. de Montréal, Montréal, QC; [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Many patterns of irrational choice can be explained by algorithmic or computational bounds. Other patterns defy psychological explanation, but could still be due to bounds on hardware: perhaps there are constraints on the brains' ability to faithfully represent the value of choice options. Here, we used methods recently developed to study the geometry of neural representations to determine (1) whether there are limits on the accuracy of the neural representation of value and (2) what impact these limits could have on rational decision-making. We trained two rhesus monkeys to perform a value-based decision-making task while we recorded in the ventromedial prefrontal cortex (vmPFC, area 14), a prefrontal region implicated in economic decision-making. The monkeys repeatedly chose from random sets of sequentially-presented options, which offered different volume of juice. We used neural state-space analyses to probe the geometry of the vmPFC population response to these offers.
Offer value representations were predictably arranged in vmPFC, but also nonlinearly: offer values traced a curved path through neuron-dimensional space. Because decoding from a curvilinear manifold becomes increasingly warped as the range of encoded items increases, the curvature predicted a specific violation of rational choice theory: that terrible offers—offers that were never chosen—should systematical affect the preference for better offers because they increased the range of offers. Indeed, monkeys were much more likely to confuse good options when an irrelevant offer was worse (and the range was bigger), compared to when an irrelevant offer was better (and the offers should have been harder to discriminate, not easier).
Theoretical work often assumes that values are linearly represented within the brain a geometry and a linear geometry is likely critical for generating rational economic decisions. However, we found that (1) value is represented nonlinearly in at least one economic decision-making region, and (2) that behavior is irrational in exactly the way we expect from this curvilinear geometry. We conclude that curvature in the value-coding manifold may be another important cause of irrational decision-making.

Disclosures: K. Jurewicz: None. **B.J. Sleezer:** None. **P. Mehta:** None. **B.Y. Hayden:** None. **B. Ebitz:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.01

Topic: H.08. Learning and Memory

Support: PAPIIT IN203918.
Conacyt 899297

Title: Ca^{2+}/calmodulin-dependent protein kinase ll alpha expression in hippocampus after moderate and intense inhibitory avoidance training

Authors: *T. A. MARIN-JUAREZ[1], C. X. RUIZ-LOPEZ[1], A. C. MEDINA[1], P. C. BELLO-MEDINA[2], G. L. QUIRARTE[1], R. A. PRADO-ALCALA[1];
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Abstract: Typical amnestic treatments are ineffective when administered to subjects trained in aversively-motivated tasks using relatively high foot-shock intensities. This effect has been found when treatments that disrupt neuronal activity are administered to different regions of the brain, including the hippocampus. However, the molecular mechanisms induced by intense training are unknown. Changes in the expression of several proteins have been associated with the consolidation process. Ca^{2+}/calmodulin-dependent protein kinase II alpha (CaMKIIα) is a protein that has been implicated in learning and memory and is a key essential mediator of synaptic plasticity. We analyzed mean fluorescence intensity (MFI) of phosphorylated CaMKIIα (p-CaMKIIα) and CaMKIIα in the hippocampus (CA1, CA3, and dentate gyrus (DG)) after moderate and intense training. Rats were trained in an inhibitory avoidance (IA) task using different intensities of foot-shock (1.0 or 3.0 mA). We also studied three controls groups: a cage group (rats that were not trained), a 0.0 mA group (rats "trained" in the task, but the foot-shock was not delivered), and a shock-only group (rats that were placed in the shock compartment and received a non-contingent 3.0 mA foot-shock). The rats from each group were sacrificed 2 or 24 h after training for immunohistochemical p-CaMKIIα/CaMKIIα protein detection. At 24h after training we found a significant high MFI ratio of p-CaMKIIα/CaMKIIα in CA3, but not in CA1 and DG, in the 1.0 and 3.0 mA groups compared with the cage control group. These results suggest that synaptic plastic changes, mediated by CaMKIIα, in this region may be required for the formation of memory of moderate and intense aversive learning. On the other hand, the fact there were no significant differences in the ratio of p-CaMKIIα/CaMKIIα expression 2 h after training, or in the other regions analyzed here, does not rule out the possibility that these regions are involved in memory formation of the IA task under both training conditions, and that there may be differential expression times depending on the regions, due to a different temporary processing of information derived from training. We thank N. Serafín, B. Osorio, E. N. Hernández, S. Méndez, A. González, and O. González for technical assistance. Supported by PAPIIT IN203918.

Disclosures: T.A. Marin-Juarez: None. **C.X. Ruiz-Lopez:** None. **A.C. Medina:** None. **P.C. Bello-Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcala:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.02

Topic: H.08. Learning and Memory

Support: PAPIIT IN203918

Title: Expression of phosphorylated CREB in striatal glial cells after moderate and intense training

Authors: *A. C. MEDINA[1], G. R. TORRES[1], J. G. TECKANI[1], P. C. BELLO-MEDINA[2], G. L. QUIRARTE[1], R. A. PRADO-ALCALÁ[1];
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Abstract: Many studies indicate that phosphorylation of cAMP response element-binding (pCREB) is related to memory consolidation, participating in genomic neuronal activity, but little is known about the involvement of glial pCREB activity in memory processes. It is known that intense training of inhibitory avoidance (IA) generates both a memory trace which is highly resistant to extinction and a protective effect against treatments that, when administered to the striatum, produce amnesia under moderate training conditions. The objective of this study was to investigate whether moderate and intense IA training would induce changes in the ratio of pCREB-positive glial nuclei in the striatum during memory consolidation. To this end independent groups of rats were trained using different foot-shock intensities (0.0, 1.0, or 3.0 mA); an additional group was kept in the bioterium and another group only received the highest electric foot-shock (3.0 mA) without being trained in the task. The brains were extracted one hour later for immunohistochemical detection of pCREB. There were no significant differences among the groups either in the dorsal or in the ventral regions. When comparing the dorsal vs. the ventral region a higher overall proportion of pCREB-positive in glial nuclei was observed in the ventral region. These findings indicate that the transcription pathway modulated by pCREB of the striatal glial cells does not participate in the consolidation of memory of intense and moderate IA training. We thank Norma Serafín, Bernardino Osorio, Nydia Hernández, Martín García, María A. Carbajo, Omar González, Ramón Martínez, and Javier Valles for technical assistance. Supported by PAPIIT IN203918.

Disclosures: A.C. Medina: None. **G.R. Torres:** None. **J.G. Teckani:** None. **P.C. Bello-Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcalá:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.03

Topic: H.08. Learning and Memory

Support: PAPIIT IN203918

Title: Creb phosphorylation in striatal neurons after moderate and intense inhibitory avoidance training

Authors: *P. C. BELLO-MEDINA[1], E. V. ALONSO-LORENZO[2], A. C. MEDINA[2], G. L. QUIRARTE[2], R. A. PRADO-ALCALÁ[2];
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Abstract: Intense training of inhibitory avoidance (IA) is brought about by increasing the foot-shook during training, which produces a strong and persistent memory that is highly resistant to extinction. Intense training protects memory consolidation against the amnestic effects of pharmacological treatments in the striatum (STR). The phosphorylation status of the cyclic AMP (cAMP)-responsive element-binding protein (CREB) is an indicator of its transcriptional activity and gene expression in memory consolidation of moderate IA training. To explore if the same happens after intense training, three groups of adult male Wistar rats were trained in IA using different intensities of foot-shock (0.0, 1.0, and 3.0 mA); two control groups were studied: one that received the higher foot-shock (3.0 mA; SO) without training, and a naïve group (Naï). These rats were sacrificed 1 h after training for immunohistochemical detection of phospho-CREB ser133 (pCREB) in the dorsal and ventral STR. The results showed an increased pCREB ratio in trained (0.0, 1.0, and 3.0 mA) and SO groups vs. the Naï group in ventral but not in the dorsal STR. In addition, the pCREB ratio showed lateralization; the right hemisphere showed a generalized increase pCREB ratio in all groups in comparison with the Naï group, while the left hemisphere showed a higher pCREB ratio in the moderate (1.0 mA) group in comparison to the other groups. A higher pCREB ratio was not found in the intense training (3.0 mA) group. The lateralization effect observed in this study is consistent with an increase in dopamine levels and D2 dopaminergic receptors in the left STR and these changes have been related to sensitivity to rewarding brain stimulation in the preferred direction of rotation, arm of a T-maze, or lever choice tasks. The increase in pCREB neurons ratio in the left STR after moderate IA training suggests that memory consolidation of moderate training induces CREB phosphorylation that

produces gene transcription related to synaptic plasticity; however, memory formation of the intense training does not activate CREB-dependent genomic mechanisms. We thank Norma Serafín, Bernardino Osorio, Nydia Hernández, Alejandra Castilla, and Ramón Martínez for technical assistence. This study was supported by PAPIIT IN203918.

Disclosures: P.C. Bello-Medina: None. **E.V. Alonso-Lorenzo:** None. **A.C. Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcalá:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.04

Topic: H.08. Learning and Memory

Support: PAPIIT IN215719

Title: Modulation of memory maintenance by calcineurin

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Abstract: Learning can be defined as the process by which an organism acquires information, while memory refers to the process of retaining that information. The latter can be divided into acquisition, consolidation, recall, and extinction phases. Nowadays, it is widely accepted that memory extinction involves the formation of a new associative memory that inhibits a previously conditioned association. Nevertheless, the cellular and molecular mechanisms that underlie these processes are still unclear. In this regard, a body of evidence suggests that the protein phosphatase calcineurin (CaN) is involved in the extinction and maintenance of some behavioral tasks. Indeed, our previous studies showed that conditioned taste aversion (CTA) extinction increases the CaN expression in the insular cortex (IC) while CaN inhibition prevents metaplastic changes produced by the extinction of CTA. CTA is a well-established learning and memory paradigm in which an animal associates a novel taste with nausea. Meanwhile, the IC is a region of the brain that lies in the temporal neocortex and is known for its role in processing aversively motivated learning tasks, like CTA. The aim of the present study was to evaluate the participation of CaN in the CTA extinction. To do so, we infused bilaterally in the IC of adult male Wistar rats, the calcineurin-specific inhibitor FK506, 30 minutes before the second extinction session. Our results show that infusion of FK506 elicits an impairment of CTA extinction. CaN inhibition slows down the extinction of CTA, thus revealing that this phosphatase plays an important role in extinction learning, and therefore in the maintenance of CTA.

Disclosures: S.E. Reyes-García: None. **M.L. Escobar:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.05

Topic: H.08. Learning and Memory

Support: PIACERI Project, University of Catania

Title: Physiological role of dopamine D3 receptors in hippocampal synaptic plasticity and memory

Authors: *M. TROPEA[1], W. GULISANO[1], A. ROMANO[1], F. GIANNINO[1], G. LEGGIO[1], D. PUZZO[1,2];
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Abstract: Dopamine D3 receptors (D3-Rs) are involved in several functions such as reward, social behavior, and movement control. Indeed, D3Rs have been proposed as a therapeutical target for drug abuse, schizophrenia, and Parkinson's disease. More recently, some studies have evidenced the involvement of D3-Rs in cognition, demonstrating that activation of D3-Rs impairs attention, working memory and episodic memory, whereas their inhibition exerts a pro-cognitive effect. However, the function of D3-Rs in physiological mechanisms underlying synaptic plasticity and memory at hippocampal level has not been thoroughly investigated. Here, we examined the role of D3-Rs in hippocampal long-term potentiation (LTP) and memory in mice. For this purpose, we first performed extracellular electrophysiological recordings at CA3-CA1 synapses of hippocampal slices treated with a D3-Rs inhibitor. Then, we investigated the effect of D3-Rs inhibition on recognition memory studied by Novel Object Recognition test on wild-type mice treated with a D3-Rs inhibitor and on D3 knock out (D3-KO) mice. We found that perfusion of hippocampal slices with a D3-Rs antagonist was able to convert early-LTP evoked by a weak tetanic stimulation into late-LTP. These effects were dependent upon protein kinase A (PKA) since a concomitant treatment with a PKA inhibitor prevented the D3-Rs inhibitor to enhance plasticity. Behavioral experiments confirmed that the pharmacological blockage or genetic deletion of D3-Rs had a cognitive enhancing effect. In fact, wild type mice treated with a D3-Rs inhibitor showed a switch from short-term into long-term memory. A similar effect was found in D3-KO animals. The analyses of discrimination index indicated that D3-KO mice were able to form long-term memory when receiving a weak stimulus compared with vehicle-treated animals that showed natural forgetting. In conclusion, our findings suggest that D3-Rs plays a role in synaptic plasticity and memory and that the improvement of memory elicited by D3-Rs antagonists can be useful to treat disease characterized by cognitive dysfunction.

Disclosures: M. Tropea: None. **W. Gulisano:** None. **A. Romano:** None. **F. Giannino:** None. **G. Leggio:** None. **D. Puzzo:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.06

Topic: H.08. Learning and Memory

Support: PAPIIT IN203918
CONACYT Grant 473131

Title: Memory consolidation and extinction after administration of cort:bsa in the inactivated dorsomedial striatum

Authors: *M. MARTÍNEZ-DEGOLLADO[1], A. C. MEDINA[1], P. C. BELLO-MEDINA[2], G. L. QUIRARTE[1], R. A. PRADO-ALCALÁ[1];
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Abstract: The consolidation process, necessary for the establishment of long-term memory, is time-dependent and supposedly requires the synthesis of proteins; some events or treatments may interfere with this process generating amnesia. We have shown that infusion of tetrodotoxin (TTX) into the dorsal striatum produces a marked impairment of consolidation of inhibitory avoidance (IA) learning and that intense training counteracts this effect. In the behavioral paradigm of IA, it has been observed that when the intensity of the aversive stimulus is increased, corticosterone (CORT) levels in blood increase after training. It has also been shown that the administration of CORT in the striatum after learning facilitates memory consolidation. It is not known, however, if in addition to a genomic action CORT exerts this facilitation by binding to neuronal membrane receptors. Therefore, it is of great relevance to determine whether activation of membrane glucocorticoid receptors by conjugated CORT (CORT:BSA) prevents the amnesic effect of intra-striatal TTX. To this end, we studied male rats bilaterally implanted with cannulae in the dorsomedial striatum (DMS), and then trained in the IA task with different foot-shock intensities (0.0, 0.5, 1.0, or 3.0 mA). Different groups received all combinations of the following treatments: vehicle (VEH) or TTX 30 min prior to training, and VEH or CORT:BSA immediately after training. Retention latency was recorded every 24 h for seven sessions to observe the processes of memory consolidation and extinction. We found that at 0.5 mA VEH-CORT:BSA treatment facilitated memory consolidation and produced a higher resistance to extinction compared to its control (VEH-VEH). At 1.0 mA, the TTX-VEH group showed amnesia and the VEH-CORT group had a higher resistance to extinction than the VEH-

VEH group, and memory consolidation was facilitated in the TTX-CORT:BSA group. Finally, at 3.0 mA, the TTX-VEH group was not amnestic, and in the VEH-CORT:BSA and TTX-CORT:BSA groups the extinction process was accelerated. In conclusion, we demonstrated that the administration of CORT:BSA in the DMS simulates the conditions of high intensity training; these results provided the first experimental evidence of a non-genomic mechanism involved in the protective effect of memory caused by intense training. We thank Norma Serafín, Martín García, Bernardino Osorio, Alejandra Castilla, Nuri Aranda, and Omar González for technical assistance. Supported by PAPIIT IN203918 and CONACYT 473131.

Disclosures: M. Martínez-Degollado: None. **A.C. Medina:** None. **P.C. Bello-Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcalá:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.07

Topic: H.08. Learning and Memory

Support: PAPIIT IN203918

Title: Blockade of striatal protein kinase c activity with chelerythrine induces deterioration of memory consolidation

Authors: *I. G. MONTIEL[1], A. C. MEDINA[1], P. C. BELLO-MEDINA[2], G. L. QUIRARTE[1], R. A. PRADO-ALCALA[1];
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Abstract: The protein kinase C (PKC) family is involved in several signaling cascades associated with different types of memory and it is suggested that its inhibition could impair memory consolidation. We decided to use a chelerythrine (CHEL), a drug that blocks all isoforms of PKC, to determine if PKC activity of dorsomedial striatum (DMS) is involved in memory consolidation of inhibitory avoidance training. In the first experiment, CHEL (3.84, 7.68 or 15.36 µg/0.5µL) was infused twenty minutes before inhibitory avoidance (IA) training using a 1.0 mA foot-shock. We found that inhibition of PKC interferes with memory in a dose-dependent manner. In the second experiment, we evaluated if CHEL would impede the formation of a short-term memory (STM). Two groups of rats were injected either CHEL or VEH 20 min before IA training and 30 min later we tested them in a STM test. We found no differences between groups and no detriment in STM. Because in the previous experiments CHEL was administered 20 min before training and retention latency was measured 48 h

afterwards, it might be argued that the amnesic effect observed was due to a state-dependent effect and not to disturbance of memory consolidation process. To evaluate this issue, in the third experiment, independent groups of rats were injected with CHEL or VEH twice: 20 min before training and 20 min before retention test. Lack of state-dependency memory was evident because CHEL produced a significant retention impairment as compared with its respective control group. We conclude that PKC in DMS participates in memory consolidation of inhibitory avoidance training probably using different molecular pathways. We thank the technical support of Bernardino Osorio, Norma Serafín, Martín García, Alejandra Castilla, Nuri Aranda and Ramón Martínez. Supported by PAPIIT IN203918.

Disclosures: I.G. Montiel: None. **A.C. Medina:** None. **P.C. Bello-Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcala:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.08

Topic: H.08. Learning and Memory

Support: University of Michigan Office of Research Award to NCT

Title: Sex differences in type I interferon modulation of learning and memory

Authors: *C. K. POSILLICO, N. C. TRONSON;
Univ. of Michigan, Ann Arbor, MI

Abstract: The neuroimmune system is necessary to carry out normal neural and cognitive functions including learning and memory, and neuroimmune activation during illness impairs these processes. There are known sex differences in the neuroimmune response to various pathogens, but whether biological sex plays a critical role in how neuroimmune activation affects learning and memory processes is unknown. Here, we investigated the interaction of neuroimmune activation and hippocampal-dependent memory mechanisms in both male and female C57Bl/6 mice using intracerebroventricular administration of a synthetic viral mimic, polyinosinic:polycytidylic acid (poly I:C), and context fear conditioning. First, poly I:C treatment induced significant cytokine responses in the hippocampus of both sexes. Males had a greater magnitude of response than females for IL-1α, IL-1β, IL-6, IL-10, IFNα, TNFα, CCL2, and CXCL10. Additionally, while both males and females showed increased expression of type I interferon β, only males showed increased type I interferon α. Next, we found that poly I:C treatment four hours prior to training in context fear conditioning resulted in significant deficits in freezing responses in both males and females when tested three days later. Pre-training poly I:C appeared to blunt training-induced cFos in the dorsal hippocampus of males, but it had no

effect on cFos levels in females. Thus, the same memory deficit in both males and females may be due to different and sex-specific mechanisms in the hippocampus. Next, we tested whether the sex difference in the type I interferon responses to poly I:C contributed to the sex difference in hippocampal mechanisms underlying memory deficits. We blocked type I interferon receptors (IFNAR) two hours prior to poly I:C treatment, followed by context fear conditioning four hours post-poly I:C. We found that IFNAR inhibition significantly rescued the poly I:C-induced deficit in males, but not females. This suggests that type I interferons play a more important role in modulating learning in males compared with females, and type I interferon signaling is a potential target for understanding sex differences in biological mechanisms of memory impairment induced by neuroimmune activation. Given that there are sex differences in the prevalence of debilitating memory disorders such as Alzheimer's disease and Post-Traumatic Stress disorder that present with neuroimmune dysfunction, it is important to identify sex-specific mechanisms of memory deficits that may lead to more effective treatments.

Disclosures: C.K. Posillico: None. **N.C. Tronson:** None.

Poster

P848. Learning and Memory: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P848.09

Topic: H.12. Aging and Development

Support: Brown Foundation

Title: Age and sex differences in the impacts of ibuprofen on cognitive functions

Authors: *K. R. MAGNUSSON, E. P. SACKINGER, J. RAUENHORST, M. FRISCHMAN, I. A. ABOU-SEADA;
Oregon State Univ., Corvallis, OR

Abstract: Epidemiological studies suggest that non-steroidal anti-inflammatory drugs reduce risk of Alzheimer's disease, but interventional studies find little benefit or negative outcomes. Our previous study showed a trend for impairment of long-term memory in aged male mice fed a low over-the-counter dose of ibuprofen (375 ppm in chow; 2.2 pills/day human equivalent). Ibuprofen also altered N-Methyl-D-aspartate (NMDA) receptor subunit expression in both young and older male mice. In the present study we examined dose response effects of ibuprofen on cognitive functions across aging in male mice and between sexes in young mice. Male and female 2-month-olds and 23-24-month old male (N=6-7) C57BL/6 mice were randomly assigned to 3 groups receiving 0, 375, or 1000 ppm ibuprofen in NIH-31M chow for 8 weeks. After 6 weeks of feeding, the Morris water maze was used to assess long-term, short-term (delayed match to place task (DMP)), and associative (cued control) memory and cognitive flexibility

(reversal trials). At the highest dose of ibuprofen, older males showed significantly poorer long-term memory, as compared to 0 ppm ibuprofen (p=.047), and there was a trend for young males to perform worse than young females at the same dose (p=.067). The young males fed 1000 ppm ibuprofen showed less perseveration for the old platform in reversal probe trials than the other doses. There were Sex (p=.029) and Age (p=021) interactions with Dose in the young only or male only analyses, respectfully, in the DMP task. The young males showed improved performance across both naïve and delayed trials when fed 375 (p =.007) or 1000 (p=.024) ppm ibuprofen, as compared to 0 ppm, suggesting that executive functions, such as search efficiency and flexibility were more improved than short-term memory. Performance in the DMP task was similar across doses for the young females and older males and all groups showed improvement in the delay trials, compared to the naïve trials. These results suggest that a high over-the-counter dose of ibuprofen (5.8 pills/day human equivalent) could impair long-term memory in older males, which could exacerbate cognitive aging and Alzheimer's disease symptoms. Ibuprofen improved cognitive flexibility and other executive functions in young males, but had little effect on young females.

Disclosures: K.R. Magnusson: None. **E.P. Sackinger:** None. **J. Rauenhorst:** None. **M. Frischman:** None. **I.A. Abou-Seada:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R21 (1R21EY030012),
Neuroscience program
College of Medicine
Department of Chemistry and Biochemistry
Field Neuroscience Institute

Title: Delivery of DNA Constructs Using Different Sized Surface Modified PAMAM Dendrimer Nanomolecules

Authors: *B. SRINAGESHWAR[1,2,3], N. SINGH[1,2], E. L. CRESPO[3], G. L. DUNBAR[1,2,4,9], D. SWANSON[5], A. SHARMA[5], U. HOCHGESCHWENDER[6,3], J. ROSSIGNOL[7,8,2];
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Abstract: The overall goal of the study is to assess the feasibility of surface modified polyamidoamine (PAMAM) dendrimer nanomolecules as a generic non-invasive molecular tool delivery platform to the brain. Dendrimers are highly branched nanomolecules that can be used to deliver drugs and biomolecules to different types of cells, including neurons, *in vitro* and *in vivo*. They are organic macromolecules with clinically favorable features such as cellular degradation, serum resistance and efficient excretion. We developed unique PAMAM dendrimers that have significantly reduced toxicity while maintaining efficient cell entry, and that have a cystamine core cleavable within cells, thereby releasing its cargo. In previous studies in HEK293 cells we achieved efficient expression of plasmid DNA using cystamine core (cys) dendrimers of generation 4 (G4) with a hydroxyl/amine ratio of 90/10, G4-cys-90/10. We systematically investigated the contribution of various parameters of dendrimer composition on transfection efficiency in primary rat neurons, using plasmid DNA expressing the fluorescent reporter EYFP under control of the ubiquitous CAG promoter. We complexed the reporter plasmid with lower generations of PAMAM dendrimers such as G1 and G2 at different ratios. While the G1 and G2 dendrimers were able to deliver the plasmid efficiently, expression was not maintained for more than a few days. We next modified the surface of the G4 PAMAM dendrimers used for forming complexes with the reporter plasmid by varying the composition of hydroxyl and amine groups (cysG4-90/10, -70/30, -50/50). We discovered that G4-70/30 dendrimers are more efficient in transforming primary neurons than G4-90/10 dendrimers, whereas G4-50/50 are toxic to neurons. Lastly, we heat-activated the G4-70/30 dendrimers at different time points prior to forming complexes with the reporter plasmid. Heat-activation for 24 hours of G4-70/30 dendrimers significantly improves transformation efficacy in neurons. Currently ongoing dendrimer modifications are aimed at increasing cargo size and biodistribution in vivo to lead to a widely useful generic non-invasive molecular tool delivery platform for the brain. Such a delivery platform would ultimately be compatible with applications in humans.

Disclosures: B. Srinageshwar: None. **N. Singh:** None. **E.L. Crespo:** None. **G.L. Dunbar:** None. **D. Swanson:** None. **A. Sharma:** None. **U. Hochgeschwender:** None. **J. Rossignol:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: The Equalizer Expression System Enables Uniform Protein Expression Levels in Transiently Transfected Cells

Authors: *S. YANG[1,4], J. YANG[7], J. LEE[5], M. LAND[2], O. A. IGOSHIN[6], F. ST-PIERRE[3]; [2]Dept. of Neurosci., [3]Neurosci., [1]Baylor Col. of Med., Houston, TX; [4]Chem. and Biomolecular Engin., [5]Systems, Synthetic, and Physical Biol. Program, [6]Dept. of Bioengineering, Rice Univ., Houston, TX; [7]Dept. of Biol. Engin., MIT, Cambridge, MA

Abstract: The functional study of natural proteins and the expression of biosensors and actuators generally relies on the expression of these transgenes from plasmids. However, transient plasmid transfections in mammalian cells produce a wide variation of plasmid copy numbers among cells, and consequently cause high expression heterogeneity. This cell-to-cell variability can result in some cells overexpressing at unphysiological or even toxic levels while others producing too low expression levels for detection or functional significance. Here, we report plasmid-based synthetic circuits - Equalizers - that compensate plasmid copy number variation at the single-cell level, thereby producing uniform gene expression in transiently transfected cells. Equalizers combine negative feedback and incoherent feedforward circuitries, enabling them to operate over a wide range of plasmid copy numbers. We demonstrate that Equalizers produce as low cell-to-cell variation as chromosomally integrated genes. We also present our ongoing attempts to produce Equalizer variants that achieve distinct expression levels. Finally, we demonstrate that episome-encoded Equalizers enable the rapid generation of extrachromosomal cell lines with stable and uniform expression for over two months. Overall, Equalizers are simple and versatile devices for homogenous gene expression and should facilitate the study of proteins, cells, and neuronal networks.

Disclosures: S. Yang: None. **J. Yang:** None. **J. Lee:** None. **M. Land:** None. **O.A. Igoshin:** None. **F. St-Pierre:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Start-up funding from the Foundation of Westlake University, National Natural Science Foundation of China grant 32050410298 to K.D.P.
2020 BBRF Young Investigator Grant to K.D.P.
MRIC Funding 103536022023 to K.D.P.
Internal grant of the National Research Center Kurchatov Institute №1056 from 02.07.2020 to F.V.S
RFBR grant №19-04-00395 to O.M.S.
RSF grant №17-14-01256 to T.V.K.
German Research Foundation (DFG, K1949/7-2) Project 241961032 to R.W.K.

Title: Rapid Directed Molecular Evolution of Fluorescent Proteins in Mammalian Cells

Authors: *S. BABAKHANOVA[1,2,3,4,5], E. JUNG[9,2], K. NAMIKAWA[10], O. SUBACH[11], D. KORZHENEVSKIY[11], T. RAKITINA[11], X. XIAO[12,13,14], D. M. COSIO[6], J. SHI[12,14,13], S. WU[12,14,13], D. PARK[3,2], L. EISENHARD[15], H. TANG[12,13,14], Y. SUN[12,13,14], R. KÖSTER[15], F. SUBACH[11], E. S. BOYDEN[7,8,2,3], K. PIATKEVICH[12,14,13];
[1]Biophysics, Stanford Univ., Stanford, CA; [2]Media Lab., [3]McGovern Inst. for Brain Res., [4]Electrical Engin. and Computer Sci., [5]Physics, [6]Brain & Cognitive Sci., [7]Dept. of Brain and Cognitive Sci., [8]Dept. of Biol. Engin., MIT, Cambridge, MA; [9]Univ. of Illinois at Chicago, Chicago, IL; [10]TU Braunschweig, Braunschweig, Germany; [11]Natl. Res. Ctr. "Kurchatov Institute", Moscow, Russian Federation; [12]Westlake Inst. for Advanced Study, Hangzhou, China; [13]Sch. of Life Sci., [14]Westlake Lab. of Life Sci. and Biomedicine, Westlake Univ., Hangzhou, China; [15]Div. of Cell. and Mol. Neurobio., Zoological Institute, Technische Univ. Braunschweig, Braunschweig, Germany

Abstract: *In vivo* imaging of model organisms is heavily reliant on fluorescent proteins with high brightness. Here we describe a practical method for rapid optimization of fluorescent proteins via directed molecular evolution in mammalian cells. We employed this approach to enhance intracellular brightness of a set of the spectrally diverse fluorescent proteins. The developed proteins demonstrated high performance for fluorescent labeling of neurons in culture and *in vivo* in model organisms such as *C.elegans, Drosophila* fruit flies, and zebrafish. The described method has great potential to be adopted by protein engineers due to its simplicity and convenience. We also believe that the new enhanced FPs will find wide application for *in vivo* imaging of small model organisms.

Disclosures: S. Babakhanova: None. **E. Jung:** None. **K. Namikawa:** None. **O. Subach:** None. **D. Korzhenevskiy:** None. **T. Rakitina:** None. **X. Xiao:** None. **D.M. Cosio:** None. **J. Shi:** None. **S. Wu:** None. **D. Park:** None. **L. Eisenhard:** None. **H. Tang:** None. **Y. Sun:** None. **R. Köster:** None. **F. Subach:** None. **E.S. Boyden:** None. **K. Piatkevich:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Whitehall Foundation

Title: Modulation of Mesolimbic Dopamine Transmission Through Selective Stimulation of Locus Coeruleus Noradrenergic Neurons.

Authors: *R. YATES, R. BHIMANI, J. PARK;
Univ. At Buffalo, Buffalo, NY

Abstract: The central catecholamines, dopamine (DA) and norepinephrine (NE), are involved in many physiological processes and behaviors ranging from fear and anxiety to learning and reward. Recent studies have suggested these processes may not be independent, as NE neurons in the locus coeruleus (LC), the primary source of NE in the brain, synapse with DA neurons in the ventral tegmental area (VTA), the major source of DA in the mesolimbic DA system. Increasing electrophysiological and pharmacological evidence has indicated a net excitatory effect of LC-NE on VTA-DA neurons via pre- and post-synaptic α-adrenergic receptors in the VTA. However, little is known about how mesolimbic DA transmission and DA-dependent behaviors are modulated directly by LC-NE through VTA-DA neurons. To better understand these anatomical and neurochemical processes, a combinatorial adeno-associated virus (AAV) approach was used to selectively target LC-NE neurons to express either a light-sensitive channelrhodopsin-2 (ChR2) or an excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADDs). Through immunohistochemistry, we showed selective expression of LC-NE terminals in the VTA. Furthermore, our data suggests that optical stimulation of these terminals in the VTA modulates DA transmission in the nucleus accumbens (NAc) of awake rats without any significant behavioral changes. Excitatory DREADD activation of LC-NE also increased electrically evoked DA release in the NAc in anesthetized rats. These fundamental studies will provide new insights to the understudied roles of direct and indirect noradrenergic modulation of dopaminergic functions using cutting edge methods (optogenetics and chemogenetics), which will delineate how NE contributes to DA-dependent learning and behaviors.

Disclosures: R. Yates: None. **R. Bhimani:** None. **J. Park:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Ablating choroid plexus epithelial cells in adult mouse brain: A new tool to approach CSF disorders.

Authors: *A. M. BEDOLLA[1], E. IWASAWA[2], A. TARANOV[1], E. FUGATE[2], D. LINDQUIST[2], F. TURCATO[1], J. WANG[1], J. GOTO[2], Y. LUO[1];
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Abstract: Cerebrospinal fluid (CSF) has multiple facets for maintaining a healthy brain. It plays a homeostatic role, regulating the distribution of substances across the parenchyma and clearing waste, and a protective role, acting as a shock absorber in the case of mechanical injury. Abnormal CSF volume is associated with developmental brain disorders, such as pediatric hydrocephalus and autism. Choroid plexus (CP) atrophy has also been described in adults with executive dysfunction. Contrary to its potential essential roles, the CP-CSF system has remained an under-explored field in neuroscience due to limitations in the number of available tools to precisely modulate development and function. Therefore, the development and characterization of robust models that allow us to manipulate CSF will have a significant impact on understanding the role of CSF in health and diseases. While exploring microglia ablation models, we unexpectedly discovered a robust tool that can break new ground for studies of the CP-CSF system in the mouse brain. Our preliminary data demonstrate that the transgenic animal with a floxed-diphtheria toxin receptor in the ROSA26 allele (iDTR) express sufficient level of DTR exclusively in the CP epithelial cells in the absence of Cre, which induces a significant loss of CSF and shrinkage of ventricular space upon diphtheria toxin (Dtx) administration. The loss of CSF and shrinkage of ventricular space are further validated by both 3D-fluid sensitive magnetic resonance imaging and histological analysis. Furthermore, TUNEL staining demonstrated specific CP epithelial cell apoptosis upon Dtx treatment in iDTR mice but not wt controls. Upon further investigation, using immunohistochemistry and a battery of behavioral assays, our data suggests disrupted CSF production and circulation leads to progressive neuroinflammation in the adult mouse brain supporting the important role of CSF in maintaining the homeostasis of the brain. We are currently evaluating the behavioral outcome of this novel CSF ablation mouse model.

Disclosures: A.M. Bedolla: None. **E. Iwasawa:** None. **A. Taranov:** None. **E. Fugate:** None. **D. Lindquist:** None. **F. Turcato:** None. **J. Wang:** None. **J. Goto:** None. **Y. Luo:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: CEPID-FAPESP, Brazil

Title: Examining the role of chromosomal structural variants in patients with severe epilepsy due to focal cortical dysplasia

Authors: *V. S. ALMEIDA[1], F. TORRES[1], M. BORGES[1], F. ROGÉRIO[2], B. CARVALHO[3], A. CANTO[1], E. GHIZONI[4], H. TEDESCHI[4], A. COAN[4], M. ALVIM[4], C. YASUDA[4], F. CENDES[4], I. LOPES-CENDES[1];

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Abstract: Examining the role of chromosomal structural variants in patients with severe epilepsy due to focal cortical dysplasia

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Introduction: Malformations of cortical development (MCD), including focal cortical dysplasia (FCD), can cause epilepsy and are often associated with refractory seizures [1]. Chromosomal structural variants have been described in patients with neurodevelopmental disorders, including intellectual disability, autism, schizophrenia, and some types of epilepsy [2-4]. We aim to investigate the presence of copy number variations (CNVs) in patients with epilepsy due to FCD.

Materials and Methods: We examined samples obtained from 30 patients with FCD. We used the Microarray CytoScanHD Affymetrix to interrogate the CNVs, which were identified using the *Chromosome Analysis Suite (ChAS)* from Affymetrix. We compared the CNVs identified in patients with control databases (BIPMed and aDGV) and databases with information about diseases and syndromes, such as OMIM, Decipher, ClinGen. Also, we used the Franklin® platform to assist in the CNV classification. **Results:** We identified six putatively pathogenic variants in five patients (16.6%). Three were losses classified as 'likely pathogenic.' The first was present in one patient at chromosome chr1p12-p11.2, involving the *NOTCH2* gene. The second was present in a single patient at chr18q21.1, involving the *SMAD2* gene. The third ‘likely pathogenic’ CNV was identified in two patients, at chr22q13.3, involving the *MLC1* gene. In addition, we found one large loss, classified as ‘pathogenic” in two patients, at chr16p13.3, and involving 13 genes, including *TSC2*.

Discussion/Conclusion: We identified CNVs with a potential role in FCD in over 16% of patients with FCD. Noteworthy, we found two patients with the same large deletion involving the *TSC2* gene. Given the frequency of putatively pathogenic CNVs found in patients with FCD, we suggest investigating these types of variants as part of the clinical workup for patients with FCD.

Disclosures: V.S. Almeida: None. **F. Torres:** None. **M. Borges:** None. **F. Rogério:** None. **B. Carvalho:** None. **A. Canto:** None. **E. Ghizoni:** None. **H. Tedeschi:** None. **A. Coan:** None. **M. Alvim:** None. **C. Yasuda:** None. **F. Cendes:** None. **I. Lopes-Cendes:** None.

Poster

P912. Genetic Techniques II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P912.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: FAPESP 2017/00648-1
CAPES 88887.508863/2020-00

Title: Investigating the burden of chromosomal structural variants in Latin American patients with developmental and epileptic encephalopathies

Authors: *H. T. MORAES[1], H. URQUIA-OSORIO[1], M. MONTENEGRO[1], A. COAN[1], M. T. MEDINA[2], R. CARABALLO[3], A. REIS[4], C. THIEL[4], S. UEBE[4], I. LOPES-CENDES[1]; [1]State Univ. of Campinas, Campinas, Brazil; [2]Natl. Autonomous Univ. of Honduras, Tegucigalpa, Honduras; [3]Hosp. JP Garrahan, Buenos Aires, Argentina; [4]Friedrich-Alexander Univ. Erlangen-Nürnberg, Erlangen, Germany

Abstract: Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of early-onset epilepsies characterized by different types of seizures associated with a high risk of global developmental delay and cognitive dysfunction. Rare copy number variations (CNVs) are known causes of the DEEs; however, information in the Latin-American population is lacking. We performed whole-exome sequencing (WES, *SureSelectXT Human All Exons V6* and *Illumina Hiseq 4000/NovaSeq 6000*) in 236 patients and chromosomal microarray analysis (CMA, *Genome-Wide Human SNP Array 6.0*) in 241 patients. From the WES data, the CNVs were called using Exome Depth 1.1.15 and were prioritized using a list of 127 genes already related to the DEEs. For the CMA data, the CNVs and the regions of homozygosity (ROH) were analyzed using the Genotyping Console Software 4.2 and BRLMM-P+ algorithm and were visualized in Chromosome Analysis Suite software 4.2. CNVs and ROH were filtered based on size, number of markers, coverage, and frequency and were classified according to criteria proposed by the American College of Medical Genetics and Genomics (ACMG). In the WES dataset, we found 11 potentially pathogenic CNVs in 11 patients (4.7%). Two of these CNVs were observed only in the WES data. In the CMA dataset, we observed 13 potentially pathogenic CNVs in 16 patients (6.6%). Eight of these CNVs were found only in the CMA data. More frequently affected chromosomes were 15 and 16, with 5 and 4 CNVs, respectively. We observed ten deletions, three duplications, one triplication, and one patient with a deletion and a duplication, both classified as pathogenic. In addition, we found large ROH in 13 patients, three of whom presented evidence of consanguinity, loss of heterozygosity in more than 5% of the genome. These regions are important targets in the search for homozygous variants responsible for autosomal recessive diseases and should be analyzed further. In conclusion, we found that rare CNVs are a significant cause of DEEs in Latin-American patients and should be investigated in the clinical setting. In addition, we found that the use of CMA combined with WES increased the chances of identifying pathogenic CNVs in these patients. Furthermore, the analysis of ROH may help identify additional causal genetic variants in patients with DEEs.

Disclosures: H.T. Moraes: None. **H. Urquia-Osorio:** None. **M. Montenegro:** None. **A. Coan:** None. **M.T. Medina:** None. **R. Caraballo:** None. **A. Reis:** None. **C. Thiel:** None. **S. Uebe:** None. **I. Lopes-Cendes:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.01

Topic: I.03. Anatomical Methods

Support: NIMH grant R21 MH099388
NIMH grant K23 MH076195
NIMH grant R01 MH110445
NIMH grant R01 MH105452
Klarman Family Foundation
Global Foundation for Eating Disorders
NIMH grant R01 MH079397

Title: The structural brain network of anorexia nervosa: results from analysis of a large, multi-study, harmonized dataset

Authors: *E. LLOYD[1,2], K. FOERDE[1,2], A. MURATORE[1,2], N. AW[1,2], D. SEMANEK[1,2], J. STEINGLASS[1,2], J. POSNER[1,2];
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Abstract: Purpose: Anorexia nervosa (AN) is characterized by persistent restrictive eating behavior, and marked fear of eating/weight-gain, despite the maintenance of a seriously low weight. Altered connectivity within structural brain networks may affect communication between diffuse brain regions, leading to abnormal cognition and behavior. Here we analyzed differences in whole-brain white matter connectivity between individuals with AN and healthy controls (HC), in a large sample. **Methods:** Diffusion-weighted MRI data acquired from females with AN (N = 147) and female HC (N = 109), aged 12-40 years, were combined across five independent studies. Probabilistic fiber tracking was completed, and full cortex connectomes describing streamline counts between 84 brain regions generated. Connectome data were harmonized, to remove scanner effects. The network-based statistic (NBS) approach tested the presence of subnetworks differing in connection strength between groups. Graph theoretical measures indicating communication/integration ability (strength and efficiency) were calculated for the 84 brain regions, and compared between groups using multiple linear regression. Associations between network and graph theory variables that differed between groups, and body mass index (BMI), were explored amongst individuals with AN using multiple linear

regression. All statistical models included age, motion, and study as covariates. **Results:** NBS outcomes indicated patients with AN, relative to HC, had reduced connectivity in a subcortical network including hippocampus, parahippocampal gyrus, thalamus, amygdala, and caudate, regions ($p < 0.05$, FWE cluster-wise corrected). NBS outcomes also indicated greater connectivity within a frontal cortical network including orbitofrontal, inferior frontal, and frontal pole, regions, among AN patients relative to HC ($p < 0.05$, FWE cluster-wise corrected). Greater connectivity within the subcortical component was related to higher BMI, while greater cortical component connectivity was related to lower BMI, in the AN group ($p < 0.05$, uncorrected). Graph theory analyses indicated greater efficiency of the left lateral orbitofrontal cortex, and reduced strength of the left hippocampus and parahippocampal gyrus, amongst AN patients relative to HC ($p < 0.05$, permutation corrected). Graph measures were not associated with BMI in the AN group. **Discussion:** Structural network differences suggesting an altered balance between cortical and subcortical connectivity are present in AN. Subnetwork connectivity is related to BMI amongst AN patients, further supporting the relevance of the structural connectome to AN.

Disclosures: E. Lloyd: None. **K. Foerde:** None. **A. Muratore:** None. **N. Aw:** None. **D. Semanek:** None. **J. Steinglass:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UpToDate. **J. Posner:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.02

Topic: I.03. Anatomical Methods

Title: Got integrity? Conventional white matter structural descriptors found to be impacted by fiber orientation dispersion across the brain.

Authors: *E. J. RIZOR[1], N. M. DUNDON[2], M. HAYES[2], S. T. GRAFTON[2];
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Abstract: Fractional anisotropy (FA), a measure commonly used to quantify white matter (WM) structural integrity, is known to be sensitive to fiber orientation dispersion. Diffusion-based q-space trajectory encoding (QTE) imaging allows for the estimation of parameters that describe diffusion tensor size, shape, and orientation (Topgaard, 2017). Independent estimations can be derived for microscopic FA (μFA) and an orientation parameter (OP) that models the degree of fiber dispersion in a voxel. The conventional DTI measure FA can be mathematically derived from these two parameters (Lasič et al., 2014). By fixing μFA or OP to 1, we can estimate their

relative contributions to FA's presumed measure of microstructure. To investigate how these contributions vary regionally across the WM, QTE images were obtained from 28 young, healthy adults (ages 18-29, 13F/15M). We hypothesized that variation of FA across the WM would be dominated by regional variation of OP, and that regions of the brain known to contain crossing fibers would be sites of increased OP domination. For each participant, images representing the percent relative difference (PRD) between FA and FA when OP is fixed to 1 (FAvFA_max_OP) and the PRD between FA and FA when μFA is fixed to 1 (FAvFA_max_μFA) were calculated. Across the WM, FAvFA_max_OP was 38.37% across all participants. In contrast, WM FAvFA_max_μFA across all participants was 6.16%. With Bayesian hierarchical modeling, hyper-distributions were generated for both PRD parameters across the deep WM and for individual parcellations as defined by the JHU ICBM WM atlas. K-means clustering analysis of these hyper-distributions revealed that regions are clustered mainly based on variation in FAvFA_max_OP. Regions in one grouping (lower FAvFA_max_OP) include the corpus callosum, the cerebellar and cerebral peduncles, and the anterior and posterior limbs of the internal capsule. Regions present in another grouping (greater FAvFA_max_OP) include the superior longitudinal fasciculus and the corona radiata. These results align with previous mappings of number of fiber orientations per voxel, where regions with greater FAvFA_max_OP are labeled as having an elevated fiber orientation count compared to others (Jeurissen et al., 2012). In conclusion, regional variation of WM FA, a descriptor of tissue integrity, is four-fold more likely to be explained by local heterogeneity of fiber orientations than by the underlying microscopic diffusion anisotropy. Conventional diffusion tensor imaging (DTI) may yield results that do not adequately reflect the underlying microstructure independent of fiber orientation.

Disclosures: E.J. Rizor: None. **N.M. Dundon:** None. **M. Hayes:** None. **S.T. Grafton:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.03

Topic: I.03. Anatomical Methods

Support: NSF GRFP

Title: Partial connectomes of adult-born granule cell dendritic integration

Authors: *A. SOROKINA[1,2], V. SAMPATHKUMAR[1,2], X. VALENCIA[1], N. B. KASTHURI[1,2];
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Abstract: The hippocampal dentate gyrus is one of few places in the mammalian brain that undergoes adult neurogenesis. While adult-born granule cells functionally integrate into the

existing circuit, the exact mechanism for how this integration occurs remains unknown. Here we used a combination of low-resolution serial electron microscopy for dendritic morphology, with targeted high-resolution serial electron microscopy for annotating synapses. We first traced the dendritic arbors of granule cells from the hilus to the outer molecular layer and classified them as adult-born, neonatal-born, or embryonic-born, based on distinct morphological characteristics. We find that synapses onto adult-born granule cell dendrites differ from synapses on neonatal or embryonic-born granule cells. Adult-born neurons were much more likely to be innervated by axonal boutons making more than one synapse, multisynaptic boutons (MSBs), than embryonic or neonatal-born granule cells. Using MSBs as a proxy for synaptic competition, our data suggests that younger cells compete with older cells for synapses in the molecular layer, supporting previous literature. Further, we find that this competition may take place on an age gradient, with older cells competing for synapses less than younger cells. We conclude that large volume electron microscopy can provide previously unknown details about how adult-born neurons integrate in the hippocampus.

Disclosures: A. Sorokina: None. **V. Sampathkumar:** None. **X. Valencia:** None. **N.B. Kasthuri:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.04

Topic: I.03. Anatomical Methods

Support: R01MH118257
P41EB027061
P30DA048742

Title: Setting the stage for chemogenetic manipulations of whole brain resting state functional connectivity in nonhuman primates

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Dept. of Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are modified G-Protein Coupled Receptors that can be targeted to select neuronal populations. Once expressed, pharmacological activation of DREADDs with a chemogenetic ligand allows for transient, remote and reversible manipulation of cells expressing the receptors. Clozapine-N-Oxide (CNO) is commonly used to activate DREADDs, however, CNO demonstrates poor brain permeability; requiring large doses to be administered and is metabolized into clozapine. Nonetheless, recent studies have shown that low dose clozapine (CLZ) and novel ligand

Deschloroclozapine (DCZ) have a higher affinity for DREADDS with minimal off-target binding.
The utilization of DREADDs in nonhuman primates (NHP), though limited in comparison to studies using rodents, allows for the causal perturbation of targeted cells, in a model species that has great homology to humans. The objective of many of these studies is to understand the function of the targeted cells/circuit, and the role they play in healthy brain functioning. Functional Magnetic Resonance Imaging (fMRI) is a non-invasive neuroimaging approach that measures fluctuation in hemodynamic signals locally and across the whole brain. fMRI has identified altered functional connectivity in many neurological and psychiatric disorders.
The aim of this study was to lay the groundwork for combining DREADDs manipulation with resting state fMRI in NHP. To this end, we first determined the infection and transport patterns of common AAV serotypes in macaques (see Cushnie et al., 2020). Next, we evaluated the effects of chemogenetic ligands on resting state functional connectivity (rs-fc) prior to DREADDs transduction in NHP, which has previously not been determined. Accordingly, macaques underwent a series of resting state fMRI scans at 10.5 Tesla. Animals were sedated, then administered intramuscularly varying doses of CLZ or DCZ (up to 0.2mg/kg) acutely before the start of each scan. Region to Region and Independent Component Analysis were used to characterize whole brain functional connectivity following administration of the ligands.
This proof of principle study revealed local and whole brain functional connectivity patterns prior to DREADDS expression, setting the stage for more detailed network interrogation using chemogenetic manipulations and rs-fMRI in NHPs.

Disclosures: A.K. Cushnie: None. **J. Zimmermann:** None. **S.R. Heilbronner:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.05

Topic: I.03. Anatomical Methods

Support: NIH Grant NS078860

Title: Alterations in intrinsic functional networks in squirrel monkey brains produced by lesions of thalamus VPL nucleus revealed using independent component analysis of resting state fMRI

Authors: A. SENGUPTA[1], A. MISHRA[1], P.-F. YANG[1], Z. YANG[2], F. WANG[1], J. L. REED[1], L. CHEN[1], *J. C. GORE[1];
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Abstract: Inter-regional correlations in resting state fluctuations of MRI signals (rsfMRI) are interpreted as indicators of functional connectivity (FC) between brain regions. Resting state functional connectivity (rsFC) has been widely used to identify and characterize functional circuits in both human and animal brains. However, the effects of deprivation of subcortical drives on rsFC and how changes in rsFC patterns are related to behavioral changes remain largely unknown. The goals of this study are to (1) quantify how interruption of thalamic inputs to cortex affect whole brain rsFC networks and (2) evaluate how changes in rsFC are related to behavioral deficits. We selectively deprived thalamic inputs to cortex by targeted lesions of the thalamus VPL nucleus and used Independent Component Analysis (ICA) to derive rsFC networks. Studies were performed on 10 healthy and (to date) one thalamus VPL lesioned monkeys at 9.4T under light anesthesia. All fMRI images were co-registered to the squirrel monkey VALIDATE atlas in order to perform group level analyses. After standard fMRI signal pre-processing steps (motion and physiological noise corrections, bandpass filtering), group spatial ICA was performed on gray matter only by temporal concatenation of all the data using GIFT software. Visual identification of the spatial locations of components revealed coherent BOLD signal fluctuations in regions and networks similar to those reported previously for non-human primates, including the visual and sensorimotor regions. A unilateral lesion was produced at the digit representation in thalamic VPL nucleus in one monkey brain using an electrode. RsfMRI data were acquired longitudinally at 1-, 3- and 10-weeks post lesion. Estimates of the correlations between different IC time series revealed significant changes in network patterns and inter-reginal connectivity strengths at different stages of recovery. Power Spectra of the IC time series also altered over time. More lesioned animals are being studied to understand how targeted lesions in the thalamus modulate specific networks in monkey brain and how these network changes predict hand sensorimotor behavioral impairments and recovery. The results from this study will provide valuable information about how synchronized BOLD signal fluctuations in cortex are driven, and confirmation of the interpretation of networks derived from rsfMRI.

Disclosures: A. Sengupta: None. **A. Mishra:** None. **P. Yang:** None. **F. Wang:** None. **J.L. Reed:** None. **L. Chen:** None. **J.C. Gore:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.06

Topic: I.03. Anatomical Methods

Support: NIH R01HL128546
NIH R01HL139712
U54 HD090257

Title: Identification of region-specific alterations in white matter microstructure and connectivity with atlas-based multilevel brain analyses in a piglet model of chronic hypoxia

Authors: *J. LI[1], C.-H. HSU[3], V. LAM[1], T. MAEDA[1], C. LEONETTI[1], G. R. STINNETT[1], K. KOBAYASHI[1], A. AGARONYAN[3], K. HAMILTON[1], K. LYDIC[1], G. TAYLOR[1], S. LIN[3], J. D. ASIS-CRUZ[1], N. SARIC[2], A. V. FARIA[4], P. C. WANG[3], T.-W. TU[3], S. MORI[4], N. ISHIBASHI[1];
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Abstract: Diffusion tensor imaging (DTI)-based brain atlases allow automatic parcellation for all brain regions including white matter (WM), facilitating inter-subject and inter-group quantitative whole brain analysis. Use of piglet brains in pediatric brain research offers various advantages due to its similarities with human pediatric brains. We created 3D piglet brain atlases with 133 cerebral structures, including 24 subcortical and 36 cortical gray matter, and 26 deep and 36 superficial white matter (SWM) regions using high-resolution DT images. Impacts of development and chronic hypoxia to WM were investigated, respectively, using multi-shell diffusion images with DTI atlas-based multilevel brain analyses, including volume, voxel-by-voxel based analysis for DTI and neurite orientation dispersion and density imaging (NODDI), structure connectomics and histological analyses. Differences in region- and tract type-dependent WM developmental changes were demonstrated with imaging and histology. Chronic hypoxia induced global brain volume reduction and WM microstructure alterations, with prefrontal-striatum connections disturbances. Following hypoxia, persistent SWM injury, with particular vulnerability in prefrontal WM, were evidenced by voxel-based analysis. Persistent demyelination and its potential association with histone modification of oligodendrocytes caused by chronic hypoxia were detected in dorsal prefrontal cortex-WM by histological analyses. Introducing DTI atlas-based multilevel diffusion imaging approach to piglet models achieves an unbiased comprehensive whole brain evaluation, with a wealth of clinical/pathological bidirectionally exchangeable information, providing a new insight into complex human brain development and a wide range of neurodevelopmental disorders.

Disclosures: J. Li: None. **C. Hsu:** None. **V. Lam:** None. **T. Maeda:** None. **C. Leonetti:** None. **G.R. Stinnett:** None. **K. Kobayashi:** None. **A. Agaronyan:** None. **K. Hamilton:** None. **K. Lydic:** None. **G. Taylor:** None. **S. Lin:** None. **J.D. Asis-Cruz:** None. **N. Saric:** None. **A.V. Faria:** None. **P.C. Wang:** None. **T. Tu:** None. **S. Mori:** None. **N. Ishibashi:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.07

Topic: I.03. Anatomical Methods

Title: Serial section electron tomography for whole-brain mammalian connectomics

Authors: A. T. KUAN[1], S. PHAN[2], M. KIM[1], M. H. ELLISMAN[2], ***W.-C. A. LEE**[3];
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Abstract: Recent advances in high-throughput electron microscopy (EM) have made it possible to image brain volumes as large as 1 mm^3 with sufficient resolution to comprehensively resolve synaptic connectivity between neurons. These approaches have produced unprecedented connectomic datasets of complete insect nervous systems and distinct microcircuits in the mouse brain. However, scaling connectomic imaging to whole-brain mouse volumes (~500 mm^3) faces critical scaling and sample preparation challenges. Here, we present a large-scale EM imaging approach that boosts effective imaging rates and circumvents the need for ultra-thin sectioning. Using intermediate voltage tomography, we show semi-thick brain sections (~1 μm) can be rapidly reconstructed at synapse resolution from a moderate number of projections (~20), and that successive sections can be stitched together to form large contiguous volumes. Furthermore, we show that the same samples can be imaged at even higher resolution using scanning transmission EM (STEM) tomography to resolve smaller structures such as gap junctions. We present a proof-of-principle volume reconstruction from the cerebellum and propose integrating electron tomography with tape-based sample handling as an end-to-end imaging solution for whole-brain mouse connectomics.

Disclosures: A.T. Kuan: None. **S. Phan:** None. **M. Kim:** None. **M.H. Ellisman:** None. **W.A. Lee:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.08

Topic: I.03. Anatomical Methods

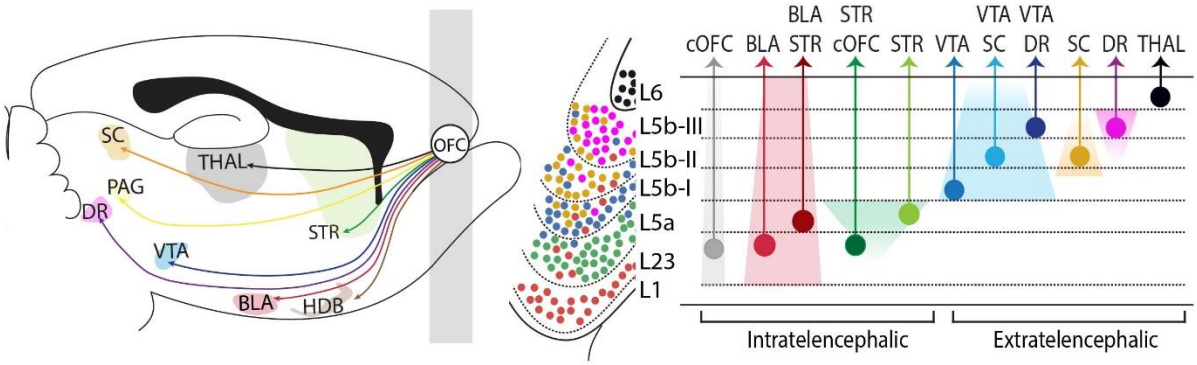
Support: NIH Grant F30

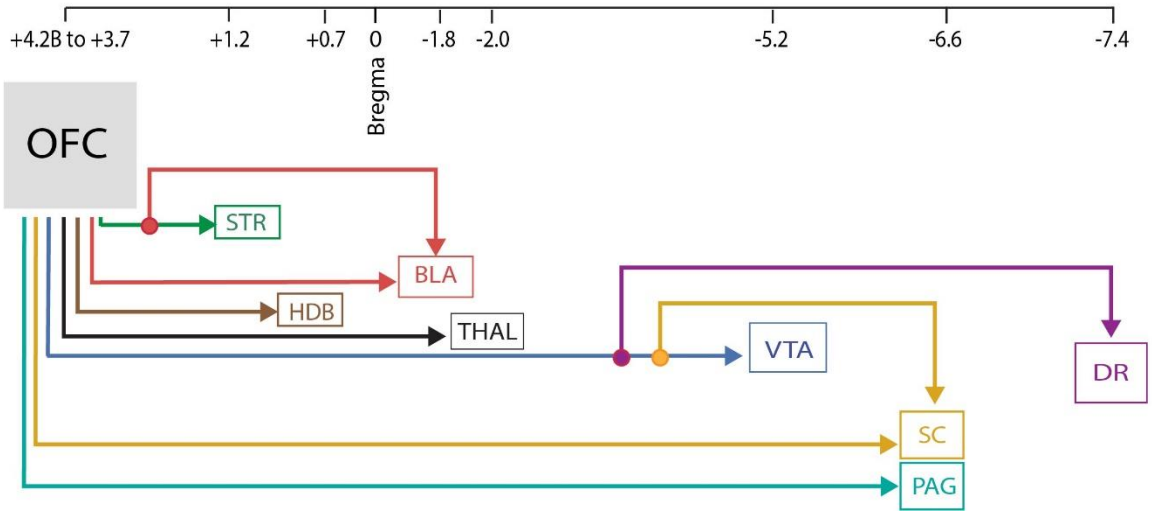
Title: Subcortical projectome of rat orbitofrontal cortex at a single neuron resolution

Authors: *S. REN[1], T. OTT[2], B.-O. NATHANSON[3], R. MUÑOZ CASTAÑEDA[4], S. LI[4], M. WU[4], S. RYU[1], J. HIROKAWA[5], T. KLAUSBERGER[3], J. TOLLKUHN[4], L. HUANG[4], A. R. NECTOW[6], P. OSTEN[4], A. KEPECS[2];
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Abstract: Orbitofrontal cortex supports economic decision making and learning through its intricate micro-circuitry. Pyramidal neurons in layer 5 constitute the main output of cortex and project outside of the cortico-thalamic system to distribute information to specialized subcortical structures, but a blueprint for the different output pathways is lacking. Here, we used a combination of viral tracing, single-cell resolution projection mapping, and RNA sequencing to comprehensively map descending outputs in rat orbitofrontal cortex - the preferred model organism to investigate complex choice behavior. At a single neuron resolution, we characterize the connectivity of ~60,000 neurons and demonstrate that most neurons project to a single subcortical target, representing a dedicated line. These connectivity-defined projection neurons preferentially reside in L5a and in intermediate sublayers of L5b, and express unique sets of genes, including transcription factors and surface receptors. Taken together, this multi-modal evidence reveals that subcortical projection neurons in orbitofrontal cortex represent genetically distinct neuronal cell types that might have distinct roles as specialized output channels.





Disclosures: S. Ren: None. **T. Ott:** None. **B. Nathanson:** None. **R. Muñoz Castañeda:** None. **S. Li:** None. **M. Wu:** None. **S. Ryu:** None. **J. Hirokawa:** None. **J. Tollkuhn:** None. **L. Huang:** None. **A.R. Nectow:** None. **P. Osten:** None. **A. Kepecs:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.09

Topic: I.03. Anatomical Methods

Support: FA9550-16-1-0410
HR0011-19-9-0035

Title: Interrogating the functional logic of Drosophila brain circuits at single-synapse scale

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Abstract: The current paradigm of computational studies of neural circuit function largely focuses on characterizing neuron connectivity. A widely-used approach is to model neurons as nodes in a graph and their connections as edges. However, this neglects the myriad of differing functions that synapses may achieve. Based upon the visualization and analysis of the Hemibrain connectome/synaptome dataset of the fruit fly brain, we propose a new computing paradigm for discovering the functional logic of fly brain circuits that shifts the modeling emphasis from communicating neurons to the interactions between blocks of synapses.
Underlying the computing paradigm introduced here, there are two building blocks: the synaptic computing complex (SCC) and the synaptic processing unit (SPU). The SCC models an axon terminal, the synapses and neuromodulatory inputs impinging on the axon terminal, and the synapse lying across active zones of the axon terminal and postsynaptic densities. SCCs can be found throughout the fly brain. The ubiquitousness of SCCs suggests that in addition to the processing in the postsynaptic neurons, computation heavily takes place in the presynaptic terminals. The SPU models a synapse-rich volume such as a glomerulus in the antennal lobe (AL) and a compartment in the mushroom body (MB). An SPU consists of SCCs as atomic building blocks and defines the computation in the SPU. The compositions of SPUs take the form of communicating neurons interpreted here as wires that connect SPUs, governing the I/O of a local processing unit (LPU) modeling a neuropil [1].
To explore the functional logic of neural circuits, we model SCCs in the AL and MB at three levels of abstraction. The first level corresponds to the ground truth synaptome of the Hemibrain dataset, where each individual axon terminal interacts with a different set of input synapses. At the second level, all the inputs onto an axon are grouped to interact with all the axon terminals of the neuron. At the third level, we group all the inputs onto the axons of neurons of the same cell

type. We provide circuit diagrams and a FlyBrainLab library to automatically map these three levels and compositions of SPUs into executable circuits by leveraging the Hemibrain dataset [2]. We quantitatively characterize the role of SCCs in shaping the response of the circuit. The I/Os of the three levels are then compared to identify the appropriate level for modeling the functional logic of each neuropil.

References
[1] Givon and Lazar, PloS one, doi: 10.1371/journal.pone.0146581, 2016.
[2] Lazar et al., eLife doi: 10.7554/eLife.62362, 2021.

Disclosures: M. Turkcan: None. **Y. Zhou:** None. **A.A. Lazar:** None.

Poster

P926. Connectomics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P926.10

Topic: I.03. Anatomical Methods

Support: NIH BRAIN Initiative RF1 MH117815-01
Intelligence Advanced Research Projects Activity (IARPA) via Department of Interior/ Interior Business Center (DoI/IBC) contract numbers D16PC00003, D16PC00004, and D16PC0005
Mathers Foundation
Google
Amazon

Title: Flywire online community for whole-brain connectomics

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Abstract: Due to advances in automated image acquisition and analysis, whole-brain connectomes with many thousands of neurons are finally on the horizon. The Drosophila community is still awaiting an adult whole-brain connectome. Despite advances in automated segmentation of EM volumes, generation of connectomes still requires human proofreading of automated reconstructions, which requires many person-years of effort, due to the huge volumes

of data involved. We created FlyWire, an online community for proofreading neural circuits in a full adult fly brain, and will discuss how its novel computational and social structures are organized to scale up to whole-brain connectomics. FlyWire is based on a previously released EM dataset (FAFB, Zheng et al., 2018), and we segmented the neurons in this dataset with high accuracy, reducing the time needed for proofreading. We also introduce a new data framework, a spatially-chunked supervoxel graph, that enables collaborative editing of neurons, making it possible to simultaneously distribute proofreading to individuals located anywhere in the world. Information in the edit history is programmatically accessible for a variety of uses such as estimating proofreading accuracy and facilitating collaboration. An open community is also accelerating scientific discovery via information sharing. We will present our progress towards generation of a fully proofread adult whole-brain connectome and demonstrate how FlyWire enables brain-wide circuit analysis.

Disclosures: S. Dorkenwald: None. **C.E. McKellar:** None. **T. Macrina:** A. Employment/Salary (full or part-time):; Zetta AI. **N. Kemnitz:** A. Employment/Salary (full or part-time):; Zetta AI. **K. Lee:** A. Employment/Salary (full or part-time):; Zetta AI. **R. Lu:** A. Employment/Salary (full or part-time):; Zetta AI. **J. Wu:** None. **S. Popovych:** None. **E. Mitchell:** None. **B. Nehoran:** None. **Z. Jia:** None. **A. Bae:** None. **S. Mu:** None. **D. Ih:** None. **M. Castro:** A. Employment/Salary (full or part-time):; Zetta AI. **O. Ogedengbe:** None. **A. Halageri:** A. Employment/Salary (full or part-time):; Zetta AI. **K. Kuehner:** None. **A. Sterling:** None. **Z. Ashwood:** None. **J. Zung:** None. **D. Brittain:** None. **F.C. Collman:** None. **C.M. Schneider-Mizell:** None. **C. Jordan:** None. **W. Silversmith:** None. **C.A. Baker:** None. **D. Deutsch:** None. **M. Murthy:** None. **H. Seung:** A. Employment/Salary (full or part-time):; Samsung, Zetta AI.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01EB026938
NIH Grant R01MH119384
NIH Grant R01NS113804
MnDRIVE Brain Conditions Initiative
McKnight Land Grant Professorship

Title: Toolkit for Oscillatory Real-time Tracking and Estimation (TORTE)

Authors: *M. SCHATZA, E. B. BLACKWOOD, S. NAGRALE, A. WIDGE;
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Abstract: Closing the loop between brain activity and behavior is one of the most active areas of development in neuroscience. There is particular interest in developing closed-loop control of neural oscillations. Many studies report correlations between oscillations and functional processes. Oscillation-informed closed-loop experiments might determine whether these relationships are causal and would provide important mechanistic insights which may lead to new therapeutic tools. These closed-loop perturbations require accurate estimates of oscillatory phase and amplitude, which are challenging to compute in real time. We developed an easy to implement, fast and accurate Toolkit for Oscillatory Real-time Tracking and Estimation (TORTE). TORTE operates with the open-source Open Ephys GUI (OEGUI) system, making it immediately compatible with a wide range of acquisition systems and experimental preparations. TORTE efficiently extracts oscillatory phase and amplitude from a target signal and includes a variety of options to trigger closed-loop perturbations. Implementing these tools into existing experiments is easy and adds minimal latency to existing protocols. Most labs use in-house lab-specific approaches, limiting replication and extension of their experiments by other groups. Accuracy of the extracted analytic signal and accuracy of oscillation-informed perturbations with TORTE match presented results by these groups. However, TORTE provides access to these tools in a flexible, easy to use toolkit without requiring proprietary software. We hope that the availability of a high-quality, open-source, and broadly applicable toolkit will increase the number of labs able to perform oscillatory closed-loop experiments, and will improve the replicability of protocols and data across labs.

Disclosures: M. Schatza: None. **E.B. Blackwood:** None. **S. Nagrale:** None. **A. Widge:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.02

Topic: I.08. Methods to Modulate Neural Activity

Support: OneMind Institute
UMN Medical Discovery Team – Addiction

Title: Frequency-dependent behavioral effects of mid-striatum deep brain stimulation on cognitive flexibility

Authors: *D. C. COOPER[1], A. E. REIMER[2], A. S. WIDGE[1];
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Abstract: High-frequency deep brain stimulation (HF-DBS, >100 Hz) has emerged as a safe and effective treatment option for motor disorders and, more recently, has shown promising results in

treatment-resistant neuropsychiatric disorders. Stimulation frequency, among other factors such as stimulation site, seems to strongly influence the motor symptoms of Parkinson's Disease. Recently, we showed that HF-DBS applied to ventral capsule/ventral striatum in patients with major depressive and/or obsessive-compulsive disorder results in improved cognitive flexibility; however, the effects of low-frequency deep brain stimulation (LF-DBS, <100 Hz) on cognitive flexibility have yet to be examined. Here, we compared the effects of LF- and HF-DBS applied to the mid-striatum of rats submitted to an operant set-shifting paradigm. Long-Evans rats (n=12) underwent bilateral mid-striatum electrode implantation followed by subsequent training. The set-shifting task consisted of daily alternate sham or stimulation treatments. Chronic biphasic stimulation (300 μA, 0.1 ms pulse width, at 130 or 20 Hz) was delivered continuously one hour prior to and while set-shifting. HF-DBS significantly reduced reaction time (β=-0.057, z=-3.01, p=0.003) without affecting total errors (p>0.05). Conversely, LF-DBS did not affect RT (p>0.05) but significantly increased total errors (β=0.193, z=2.19, p=0.029). Our study shows that frequency is an important parameter in accessing the effects of mid-striatum DBS on cognitive flexibility.

Disclosures: D.C. Cooper: None. **A.E. Reimer:** None. **A.S. Widge:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.03

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant MH113103
OneMind Institute
UMN Medical Discovery Team - Addiction

Title: Behavioral Effects of Mid-Striatum Deep Brain Stimulation Reflect Cognitive Flexibility, Not Impulsivity

Authors: *A. J. WALD[1], A. E. REIMER[2], A. ALPERS[3], D. COOPER[1], E. HOSKINS[1], K. SINGH[1], K. WARNER[1], A. S. WIDGE[1];
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Abstract: Abstract Deep brain stimulation (DBS) has emerged as a safe and effective treatment option for motor disorders and, more recently, has shown promising results in treatment-resistant neuropsychiatric disorders. Recently, we showed that DBS applied to patients with major depressive and/or obsessive compulsive disorder results in improved cognitive flexibility. We replicated this in rodents, where DBS of the mid striatum significantly reduced reaction time in a

Set-Shifting task ($z= -3.01$, $p = 0.003$) without affecting total errors ($p > 0.05$), however, the mechanism behind this improvement has yet to be determined. We needed to decipher whether or not the improvement seen in this previous study reflected an increase in cognitive flexibility, or an increase in impulsive behavior. An increase in cognitive flexibility would give back a patient's ability to live their lives without the tendancy toward risky behavior which may come with an increase in impulsivity.To do so, we submitted Long-Evans rats to a 5 Choice serial reaction time (5CSRTT) paradigm. This task is designed to measure impulsive-like behavior which is one potential explanation for the improvement seen in the set-shifting task. Rats were trained on the task and then underwent bilateral electrode implantation targeting the mid-striatum. All animals performed the 5 choice task daily, with sham and active stimulation given on alternating days. For the stimulation treatment, biphasic stimulation (300 µA, 0.1 ms pulse width, at 130 Hz) was delivered continuously, one hour prior to and during the 5 choice task. This stimulation did not result in a significant change in number of premature responses ($z = 1.29$, $p = 0.198$) or number of correct responses ($z = -0.223$, $p = 0.824$). Our study suggests that the effect of stimulation to the mid-striatum reflects a true increase in cognitive flexibility, and cannot be explained by an increase in impulsive responding.

Disclosures: A.J. Wald: None. **A.E. Reimer:** None. **A. Alpers:** None. **D. Cooper:** None. **E. Hoskins:** None. **K. Singh:** None. **K. Warner:** None. **A.S. Widge:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.04

Topic: I.08. Methods to Modulate Neural Activity

Support: Supported by NIMH grant MH119384

Title: Establishing an Avoidance Task to Study Pre-frontal Cortex and Amygdala Interactions

Authors: S. J. OLSON[1], V. PATEL[2], A. J. WALD[3], S. S. NAGRALE[3], *D. J. TITUS[1], A. S. WIDGE[4];
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Abstract: The ability to discriminate between threatening and non-threatening environmental cues and execute an appropriate behavioral response is crucial for survival and for maintaining a healthy balance between avoidance and reward seeking. However, little is known about the neural circuits that underlie this decision making. The basolateral nucleus of the amygdala (BLA), infralimbic (IL) and prelimbic (PL) medial prefrontal cortices have been implicated in

reward-seeking and fear-related responses, and often have altered outputs in psychiatric disorders like anxiety and Post Traumatic Stress Disorder (PTSD). Little is known about the circuit-level connectivity between these regions that coordinates appropriate behavioral responses. Platform mediated avoidance (PMA) is a behavioral paradigm that resembles real life avoidance scenarios and captures approach-avoidance conflict behavior, which is critical for its application to clinical avoidance. In this paradigm, rats learn to avoid a tone-signaled footshock by stepping onto a platform, with the cost of avoidance being the loss of access to food.
This study aims to explore interactions between the medial pre-frontal cortex (mPFC) and amygdala in behaving animals during the PMA task. Adult Long-Evans rats (n=6) between 5 and 7 months of age received electrode bundles or tetrode implants in the IL and PL of the mPFC and BLA regions after training in the PMA task. Following 20 days of high conflict training, 3 of 6 trained rats spent more time in the platform zone (exhibiting more avoidance like behavior), one rat spent more time in the reward zone (exhibiting more reward seeking behavior) and two rats timed appropriately to balance reward seeking and avoidance of the foot shock.
We will analyze the correlation between the oscillatory synchrony in theta frequency band (4-8Hz) between IL to BLA, and PL to BLA, and behavioral responses such as time spent in different zones (platform zone, reward zone and out of platform zone) or bar press rates during PMA high conflict performances. This study is an exploratory study that may provide a neurobehavioral correlation during motivated decision making. The outcome of this study could allow us to find specific targets that are altered in anxiety disorders like PTSD and serve as a basis to develop stimulation-based therapeutic interventions for these disorders in humans.
Supported by NIMH grant MH119384.

Disclosures: S.J. Olson: None. **V. Patel:** None. **A.J. Wald:** None. **S.S. Nagrale:** None. **D.J. Titus:** None. **A.S. Widge:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.05

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R01MH119384-01
Boston University 4500002985

Title: Alteration of state dependent brain connectivity using a closed loop stimulation method in a fear regulation circuit

Authors: *R. YOUNK, D. J. TITUS, S. NAGRALE, K. DRAHOS, M.-C. LO, A. WIDGE; Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Information flow in brain networks may depend on the oscillatory synchrony of local field potential (LFP) between regions. For fear-related disorders, the connection between the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) is particularly important. Theta-band (4-8 Hz) PFC-BLA LFP synchrony correlates with the ability to learn and recall safety memories, but acute stressors alter the oscillatory synchrony between these regions. Manipulating that synchrony may thus require strategies that adapt to changes in the circuit biology. We sought to assess an adaptive stimulation method to alter PFC-BLA connectivity based on stimulation locked to the theta oscillation. Electrodes with 8 recording channels and one stimulating channel were implanted into infralimbic cortex (IL, the rodent homologue of mPFC) and BLA of Long-Evans rats. Rats underwent 16 phase-locked stimulation sessions. Single electrical pulses (90 μs and 100 μA) were delivered to BLA when IL phase was at 0, 90, 180, or 270° at theta band for a total of 4 sessions per phase. Each session consisted of pre recordings and evoked response potential (ERP) measurements (50 biphasic pulses, 90 μs and 100 μA pulse width, delivered to either IL or BLA), followed by 30 minutes of phase-locked stimulation, and 60 minutes of post recordings with ERP measurements every 15 minutes. Rats then underwent a three-day tone-shock and extinction paradigm with habituation/conditioning, extinction, and recall phases. After the recall phase, rats underwent 16 phase-locked stimulation sessions as previously described. We measured stimulation induced LFP connectivity changes for targeted phase and pre/post conditioning. Prior to conditioning, we expect that 180-degree phase-locked stimulation will be most effective in increasing LFP synchrony and enhancing synaptic connectivity in the IL-to-BLA direction. Since phase-locked stimulation is concordant with the brain's natural rhythm, we also expect stimulation to continue to induce LFP synchrony post fear conditioning, although it is unknown which phase will be optimal. In summary, we have proposed a phase-locked electrical stimulation technique to alter brain oscillatory synchrony pre and post tone-shock conditioning and are exploring that technique's state dependence relative to emotionally valenced learning (shock conditioning). If we can identify methods for manipulating IL-BLA connectivity that are viable even after acute stressors, these may lead more effective therapies for treatment-resistant varieties of psychiatric disorders.

Disclosures: R. Younk: None. **D.J. Titus:** None. **S. Nagrale:** None. **K. Drahos:** None. **M. Lo:** None. **A. Widge:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.06

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH NINDS BRAIN Initiative Grant 1UH3NS103549
NIH NINDS F99 Fellowship F99NS124181

Title: Deep brain stimulation modulates oscillatory dynamics in cognitive control in treatment-resistant depression

Authors: *A. ALLAWALA[1], S. VARTANY[2], J. ADKINSON[5], H. RITZ[3], R. MATHURA[5], D. OSWALT[7], K. BIJANKI[5], A. SHENHAV[4], W. GOODMAN[6], N. POURATIAN[8], D. A. BORTON[1], S. A. SHETH[5];
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Abstract: Major Depressive Disorder (MDD) is one of the most common mental disorders in the US and the leading source of disability worldwide. Cognitive control has been shown to be impaired in depression, manifesting as rumination, negative automatic thoughts, and biases. Deep brain stimulation (DBS) has shown promise for treating treatment-resistant depression (TRD) and enhancing cognitive control, but the implicated cognitive control neurophysiological network and how it is modulated by DBS remains unclear. Here we report the outcome from a parametric cognitive control task performed in subjects with TRD (N=2) and refractory epilepsy (N=6) in an in-patient hospital setting with continuous intracranial electrophysiological monitoring. Both TRD and Epilepsy Cohorts were implanted with temporary sEEG electrodes across regions, including the dorsolateral prefrontal cortex (dlPFC), dorsal anterior cingulate cortex (dACC) and medial orbitofrontal cortex (mOFC). The TRD Cohort was also implanted with DBS leads in the ventral capsule/ventral striatum (VC/VS) and the subcallosal cingulate (SCC). In the task, a cloud of colored dots moving left or right is presented on a computer screen. Subjects were asked to indicate the color of the dots(relevant feature) by pressing a left or right button, while ignoring the motion direction(distracting feature).The congruence of motion direction and the required response side was varied on a continuous scale, allowing for parametric control of conflict level for each trial. The task was performed pre-, post-, and during DBS in TRD participants. Across both cohorts, sEEG recordings from dlPFC, dACC, and mOFC showed that activity in the theta (4-7 Hz) and high gamma (80-150 Hz) bands were parametrically modulated with task conflict. Stimulation at 130 Hz in the VC/VS and SCC improved task accuracy (42% increase overall for the first participant) and reduced reaction time (300 ms decrease overall for the second participant). An increase in theta power across contacts in the PFC and ACC in the first participant was observed during DBS alongside differences in spatial distribution of contacts exhibiting theta band power from pre to post-DBS states. Epilepsy participants showed comparable reaction times (1100 ms overall ±161 ms SEM) and accuracy (80% overall ±8%SEM) to pre-DBS TRD participant task performance. Further work will compare changes in spectral power between both Cohorts as a function of conflict, as well as during DBS for the second TRD participant. These recordings, coupled with causal manipulation will help shed light on the neurophysiology of controlled decision-making and possible mechanisms of therapeutic action of DBS.

Disclosures: A. Allawala: None. **S. Vartany:** None. **J. Adkinson:** None. **H. Ritz:** None. **R. Mathura:** None. **D. Oswalt:** None. **K. Bijanki:** None. **A. Shenhav:** None. **W. Goodman:** None. **N. Pouratian:** None. **D.A. Borton:** None. **S.A. Sheth:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.07

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF EEC-1028725

Title: Orientation and amplitude of directional deep brain stimulation impact evoked potentials at sensorimotor cortex

Authors: *C. PASCHALL[1], L. LEVINSON[1], S. SUN[2], J. G. OJEMANN[3], A. KO[4], J. A. HERRON[5];
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Abstract: Directional Deep Brain Stimulation (dDBS) uses radially segmented electrodes to direct current to a targeted volume of neural tissue with more precision than traditional DBS. Stimulation of subcortical targets using the cylindrical electrodes of traditional DBS has been shown to evoke potentials in contralateral sensorimotor cortex which modulate with the amplitude of depth stimulation. Our study characterizes the effect of stimulation orientation in directional DBS on both contralateral and ipsilateral cortical evoked potentials. To do this, neural data were collected intraoperatively from patients undergoing Boston Scientific Vericise dDBS implantation to treat Parkinson's Disease (n=6) and Essential Tremor (n=2). The Vericise probe offers a 1-3-3-1 configuration of cylindrical (1) and radially segmented (3) electrodes. Single, constant current, biphasic stimulation pulses of 200us per phase were delivered for 2 minutes at a rate of 2Hz, yielding 240 pulses with approximately 500ms of post-stimulation evoked potential recording, across each multipolar configuration of the six radially segmented electrodes. Both quadripolar and bipolar electrode configurations at four stimulation amplitudes of 2,3,4 and 5mA were explored. An example quadripolar configuration is one in which vertically aligned electrodes 2 and 5 serve as the positive pole and electrodes 3 and 6 the negative pole, while an example bipolar configuration would be between electrodes 2 and 5. The vertically-aligned quadripolar stimulation pair approach limits depth stimulation to 3 distinct orientation combinations: 2/5 and 3/6, 2/5 and 4/7, and 3/6 and 4/7, while the bipolar configurations offer 15 potential (6-choose-2) combinations. To evaluation evoked potentials, windowed root mean square (rms), peak-to-trough magnitude and latency, and high-gamma band power metrics were implemented, and the distributions compared in a multi-way ANOVA to evaluate the variance in

each EP metric attributable to stimulation orientation, magnitude, and the interaction of orientation and magnitude. Our analysis reveals that evoked potential (EP) rms magnitude and high-gamma power modulate with stimulation orientation and magnitude. Using alignment of preoperative MRI and intraoperative CT, EPs were shown to be most pronounced and modulated in cortical electrodes associated with the primary sensorimotor cortices. Intraoperative EPs may predict the clinical efficacy of directional stimulation and may guide the selection of dDBS therapeutic parameters.

Disclosures: C. Paschall: None. **L. Levinson:** None. **S. Sun:** None. **J.G. Ojemann:** None. **A. Ko:** None. **J.A. Herron:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.08

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R01DA033641

Title: Differential Effects of Nucleus Accumbens Deep Brain Stimulation on Cocaine Reinstatement in Female and Male Rats

Authors: *S. T. MANKAME, S. J. WOROBEY, S. E. SWINFORD-JACKSON, R. C. PIERCE;
Dept. of Psychiatry, Robert Wood Johnson Med. School, Rutgers Univ., Piscataway, NJ

Abstract: There are currently no FDA approved treatments to reduce cocaine craving and relapse. Recent basic and clinical studies have shown that deep brain stimulation (DBS) in limbic regions of the brain involved in drug craving, particularly the nucleus accumbens, can reduce drug seeking behavior. Previous work from our laboratory and others indicated that DBS of the nucleus accumbens shell attenuated cocaine reinstatement in male rodents. Female subjects have not yet been assessed in experiments of this sort, which is a significant oversight since it is becoming increasingly evident that biological sex is an important variable to consider when it comes to drug seeking behavior. A growing body of evidence indicates that female rodents are more susceptible to the reinforcing effects of cocaine and therefore more vulnerable to relapse during the estrus phase. Here, we sought to determine the effect of nucleus accumbens shell DBS on drug seeking behavior in female rats, and whether this effect is dependent on estrous cycle phase. Female rats were allowed to lever press for i.v. cocaine two hours daily for 21 days under a fixed ratio 1-5 schedule. Responding on the active lever resulted in an infusion of cocaine while the inactive lever presses had no consequences. Rats then underwent extinction where responses on the active lever produced infusions of saline. Daily extinction sessions continued

until lever pressing fell below 20% of responding under cocaine. Prior to any reinstatement sessions, the rats were divided into two groups to either be tested in the estrus or the non-estrus phases; each group received both DBS and sham stimulation during two counterbalanced reinstatement sessions. Cocaine seeking behavior was reinstated with an intraperitoneal injection of cocaine (10 mg/kg). Prior to the priming injection of cocaine, the nucleus accumbens shell was electrically stimulated with either 0 µA or 150 µA delivered at 160 Hz throughout the two-hour test session under extinction conditions. Preliminary data suggest that, contrary to the attenuation of cocaine seeking in male rats, DBS of the nucleus accumbens shell does not attenuate and may even enhance cocaine seeking behavior in female rats. Ongoing behavioral experiments will further clarify the potential role of the estrous cycle in the effect of DBS on cocaine seeking in female rats. Our initial results indicate clear sex differences in the effects of DBS on cocaine priming-induced reinstatement of drug seeking in rats.

Disclosures: S.T. Mankame: None. **S.J. Worobey:** None. **S.E. Swinford-Jackson:** None. **R.C. Pierce:** None.

Poster

P980. Electrical Methods to Modulate Neural Activity IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P980.09

Topic: I.08. Methods to Modulate Neural Activity

Support: National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number NS092730
Farmer Family Foundation (Funding)
Abbott/St Jude (equipment and materials)

Title: Detection of deep brain activities via cortical magnetoencephalography of deep brain stimulation

Authors: *J. BORE;
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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a well-established treatment approach for patients with Parkinson's disease (PD). The success of DBS is highly dependent on delivering the appropriate dose of stimulation at the best location within the target region. The STN is subdivided into different sub-territories (motor, associative, and limbic), each with different connections and specific functions. The most effective contacts to ameliorate PD symptoms segregate to the dorso-lateral, sensorimotor aspect of the STN, whereas current spread to the limbic and associative sub-segments may be linked to the affective and cognitive side-effects. Methods: In this study we stimulated different sub-regions of the STN and evaluated the

cortical effects using magnetoencephalography (MEG)-derived source imaging in PD patients. In particular, we performed source localization to characterize the neural sources of activity within the brain of a PD patient, and the effects of subthalamic nucleus (STN) DBS on these sources. Results: Our findings from DBS of distinct regions of STN support the segregated model of the basal ganglia (BG), which describes the anatomical separation of sensorimotor, limbic, and associative sub-regions. In particular, activation of the directional DBS contacts in the of sensorimotor area of the STN reveal sources in the motor cortex, while activation of the DBS contacts in the associative regions localized neural sources in the frontal brain areas. Conclusion: These findings show the capabilities of MEG to detect and differentiate cortical activities during DBS. Significance: DBS lead diameter and electrode recording configurations can affect the post-implant interpretation of oscillatory features. Such insights will be important for extrapolating results from intraoperative and externalized LFP recordings.

Disclosures: J. Bore: None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.01

Topic: B.06. Synaptic Transmission

Support: NIH Grant MH086507
NIH Grant MH105488

Title: Manipulation of local GABAergic interneurons disrupts prefrontal cortex dependent behaviors

Authors: *A. OROZCO, E. HU, S. DUBE, A. CABALLERO, K. Y. TSENG;
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Abstract: A variety of complex functions such as working memory, learning, and emotional regulation are largely dependent on intact prefrontal cortex (PFC) function which is determined by a careful balance of excitation and inhibition. This balance is set by the activity of excitatory pyramidal neurons and inhibitory GABAergic interneurons. Previously published data indicate that post-synaptic blockade of GABA transmission in the PFC disrupts many PFC-dependent behaviors. However, within the PFC, it remains unclear how interactions between local GABAergic interneurons and pyramidal cells ultimately affect PFC function to elicit biologically relevant behaviors. To address this, GABAergic activity in the PFC was manipulated in adult rats using designer receptors exclusively activated by designer drugs (DREADD) to either stimulate (Gs) or inhibit (Gi) local GABAergic interneurons in the adult PFC. Assessment of PFC functionality was determined through a two-day trace fear conditioning and extinction paradigm,

in which the extinction phase is strongly dependent on PFC activity. The Gs and Gi DREADDs were activated by clozapine N-oxide (CNO) 20 minutes before the extinction phase to determine whether it is altered by excitation or inhibition of prefrontal GABAergic interneurons. Our results revealed that rats who received the Gs DREADD treatment had a decreased rate of extinction compared to rats who received Gi DREADD treatments. In other words, Gs DREADD treated rats displayed more extensive prefrontal deficits. These results suggest that in normal animals, increasing GABAergic activity is disruptive to PFC function, and thus could have potentially detrimental effects throughout other areas of the brain modulated by the PFC.

Disclosures: A. Orozco: None. **E. Hu:** None. **S. Dube:** None. **A. Caballero:** None. **K.Y. Tseng:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.02

Topic: B.06. Synaptic Transmission

Support: National Institute of Mental Health Intramural Research Program
Brain and Behavior Research Foundation NARSAD Young Investigator Award to HAT
NIH Center for Compulsive Behaviors Fellowship to HEY

Title: Kappa Opioid receptor control of prefrontal cortex circuitry

Authors: *H. E. YARUR, S. M. CASELLO, H. WANG, S. MALDONADO, Q. WANG, H. A. TEJEDA;
Unit on Neuromodulation and Synaptic Integration, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The prefrontal cortex (PFC) is a key brain area implicated in the processing and regulation of fear memories, which are due to an exposure to a stressful stimulus. In stressful/aversive situations opioidergic neurotransmission is increased in a variety of limbic nuclei. Yet, the underlying neurobiological principles of opioidergic neurotransmission during aversive experiences are still unknown. Kappa opioid receptor (KOR) is component of the opioid system which controls pain, reward and addictive behaviors. This system is composed of three receptor subtypes, of which KOR activation by endogenous DYN or exogenous agonists induces negative effects in humans and animals. KORs are expressed in brain areas implicated in aversive learning in both humans and rodents, such as the PFC. DYN is highly expressed in the prefrontal cortex of humans and rodents. We hypothesize that PFC DYN/KOR system activity regulates processing of aversive information by modulating PFC circuitry. Unpublished work from our laboratory has demonstrated that DYN is expressed in both excitatory and a subset of

inhibitory neurons in the PFC. However, there is a critical knowledge gap in our understanding of the role of the endogenous DYN/KOR systems in regulating prefrontal cortical circuits. To this end we performed RNA scope, whole cell patch clamp recordings and tracing experiments in transgenic mice to disentangle the role of KOR in the control of PFC circuitry. Our results demonstrate that the PFC is innervated by several KOR-expressing inputs, including the basolateral amygdala (BLA) paraventricular nucleus of the thalamus (PVT), and claustrum. a. KOR expression in PFC neurons is primarily localized to excitatory neurons in deeper layers, with extremely sparse expression in inhibitory interneurons. KOR-expressing synapses within the PFC are inhibited by dynorphin via KORs. Importantly, KORs do not regulate all PFC afferents as ventral hippocampal inputs are insensitive to dynorphin regulation and do not express KORs. Our results suggest that KORs exert control over PFC circuitry by regulating afferent drive in a pathway-specific manner. This study increases our understanding of the neuronal mechanisms that underlay psychopathologies wherein processing of aversive experiences is dysregulated, like in anxiety and mood disorders, and provide a novel therapeutic for their treatment.

Disclosures: H.E. Yarur: None. **S.M. Casello:** None. **H. Wang:** None. **S. Maldonado:** None. **Q. Wang:** None. **H.A. Tejeda:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.03

Topic: B.06. Synaptic Transmission

Support: NIH/NIAAA Grant AA025110
NIH/NIAAA Grant AA010761
NIH/NIAAA Grant AA027330

Title: Divergent roles of the medial PFC and the mediodorsal thalamus in alcohol-related behaviors

Authors: *J. A. RINKER[1], J. KNAPP[1], R. D. CANNADY[3], S. N. ROBERTS[1], H. C. BECKER[2], J. J. WOODWARD[1], P. J. MULHOLLAND[1];
[1]Neurosci., [2]Psychiatry, Med. Univ. of South Carolina, Charleston, SC; [3]Biol., NC A&T State Univ., Greensboro, NC

Abstract: Alcohol use disorder (AUD) is a chronic and relapsing condition characterized by uncontrolled, excessive alcohol consumption. Adaptations in medial prefrontal cortical (mPFC) networks produced by chronic alcohol exposure can enhance incentive salience of cues associated with alcohol and diminish engagement in behaviors related to alternative rewards.

There is a substantial gap in our understanding of the mechanisms and neurocircuitry underlying this cortically driven inflexible behavior. The mediodorsal thalamus (MD) is a higher-order thalamic nucleus that is reciprocally connected to the mPFC, and acts as hub for integration of cortical and subcortical signaling. The MD plays a critical role in adaptive goal-directed choice behavior and higher-order cognitive flexibility, making it perfectly situated to play a central role in mediating cortical deficits in AUD, and yet has gone largely unexplored in the context of alcohol-related behaviors. These experiments used a combination of alcohol drinking and dependence models coupled with ex vivo slice electrophysiology, immunolabeling, and in vivo calcium imaging to study activated cortical and thalamic neurons. As well, use of the newly developed Targeted Recombination in Active Populations (TRAP) mouse line (FosTRAP) that allows tamoxifen-inducible Cre expression of tdTomato in *Fos*-expressing cells in a temporally controlled manner with slice electrophysiology allowed identification of functional adaptations in alcohol-activated neuronal ensembles. Chronic intermittent ethanol (CIE) induced different patterns of cFos activation during acute withdrawal in the mPFC and the MD, as well as different CIE-induced changes in evoked-firing. While both regions showed increases in tdTomato+ cells in the mPFC, as well as the MD thalamus, patch-clamp electrophysiological recordings demonstrated that tdTomato+ in mPFC layer II/III, which receives dense innervation from the MD, fired more evoked action potentials than adjacent non-activated neurons. And finally, the mPFC and the MD show divergent patterns of calcium-dependent neural activity during active and voluntary alcohol consumption. Findings from these studies using cutting-edge technology in mice identified stable and specific subsets of neural populations in cortical and thalamic circuits that are activated by alcohol drinking and dependence. The functional assessment revealed neural mechanisms and functional signatures underlying ethanol-induced plasticity that may drive heavy drinking and loss of reward-based flexible behaviors in individuals with AUD.

Disclosures: J.A. Rinker: None. **J. Knapp:** None. **R.D. Cannady:** None. **S.N. Roberts:** None. **H.C. Becker:** None. **J.J. Woodward:** None. **P.J. Mulholland:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.04

Topic: B.06. Synaptic Transmission

Support: NIH Grant MH116445
NIH Grant MH114170
NIH Grant MH122228
NIH Grant MH115688

Title: Ensemble encoding of conditioned fear by prefrontal somatostatin interneurons

Authors: *R. CLEM[1], K. CUMMINGS[2], P. KENNY[2], S. BAYSHTOK[2];
[1]Mount Sinai Sch. of Med., New York, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Our conceptual understanding of memory encoding places a great deal of emphasis on the recruitment of sparse neural ensembles, sometimes referred to as "engram cells", whose reactivation during recall plays an important role in memory expression. Trace formation is primarily assumed to be the function of excitatory projection cells that populate these ensembles. However, the cellular composition of memory-related ensembles remains largely unexplored, and therefore the contribution of discrete cell types is in fact poorly understood. Here we show that fear conditioning activates a heterogeneous neural ensemble in the medial prefrontal cortex (mPFC), comprised to a large degree of GABAergic interneurons immunoreactive for somatostatin (SST-INs). Using intersectional genetics in combination with electrophysiological and optogenetic approaches, we found that fear learning-activated SST-INs exhibit distinct circuit properties, are preferentially reactivated during memory retrieval, and mediate the expression of defensive freezing. We further established that a rewarding experience, morphine treatment, activates an orthogonal SST-IN population that exerts opposing control over fear. Overall, our results point to an unappreciated capacity for functional specialization among SST-INs and suggest microcircuit mechanisms by which they orchestrate selective control over excitatory populations. In particular, these findings suggest that SST-INs coordinate the firing of memory-related projection neurons as part of a functional "subnetwork", which has important implications for stimulus processing by mPFC under normal and pathological conditions.

Disclosures: R. Clem: None. **K. Cummings:** None. **P. Kenny:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); P.J.K. is co-founder and shareholder in Eolas Therapeutics Inc., which has a licensing agreement with AstraZeneca to develop a novel therapeutic for the treatment of substance use disorders.. **S. Bayshtok:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.05

Topic: B.06. Synaptic Transmission

Support: NIAAA R21AA028088-01
Brain and Behavior Research Foundation NARSAD Young Investigator Award

Title: Prelimbic somatostatin neurons control binge drinking

Authors: *N. CROWLEY;
Biol., Penn State Univ., University Park, PA

Abstract: Somatostatin (SST) neurons have been implicated in a variety of neuropsychiatric disorders such as depression and anxiety, but their role in substance use disorders, including alcohol use disorder (AUD), is not fully characterized. In addition, little work has been done to explore the role of SST peptidergic signaling in behaviors related to substance use. Here, we found that repeated cycles of alcohol binge drinking via the Drinking-in-the-Dark (DID) model led to hypoactivity of SST neurons in the prelimbic (PL) cortex. We found that both DREADD-induced excitation and silencing of PL SST neurons reduced alcohol binge drinking in males and females. In addition, we established that alcohol binge drinking disinhibited pyramidal neurons by augmenting SST neurons-mediated GABA release and synaptic strength onto other GABAergic populations and reducing spontaneous inhibitory transmission onto pyramidal neurons. Pyramidal neurons additionally displayed increased intrinsic excitability. Direct inhibition of PL pyramidal neurons via DREADD activation was sufficient to reduce alcohol binge drinking. Together these data revealed an SST-mediated microcircuit in the PL that modulates the inhibitory dynamics of pyramidal neurons, a major source of output to subcortical targets to drive reward-seeking behaviors and emotional response. Our next step was to characterize the role of SST peptide signaling in this circuit. Bath application of physiologically-relevant concentrations of SST reduced the intrinsic excitability of pyramidal neurons in both male and female PL layer 2/3 pyramidal neurons, supporting the role of this peptide as a mechanism to dampen PFC output activity. This effect was more pronounced in pyramidal neurons projecting to the nucleus accumbens, as compared to those projecting to amygdalar regions, suggesting a key role in modulating reward circuitry. We then further investigated whether administration of SST receptor targeting compounds themselves could augment behaviors related to anxiety- and depression. Using an SST analogue, we show that modulating SST activity directly in the PL cortex reduces anxiety-like behavior, with clear implications for alcohol use. Overall, we provide a mechanistic role for both SST neurons and SST peptide signaling in PL circuit function and AUD.

Disclosures: N. Crowley: None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.06

Topic: E.02. Cerebellum

Support: European Union’s Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)

Title: Combined dopaminergic and gabaergic regulation of prefrontal cortex activity by the cerebellum in anesthetized mice

Authors: *D. DI DOMENICO, I. MONTAGNA, L. MOSCATO, S. TRITTO, L. MAPELLI, E. D'ANGELO;
Dep. of Brain and Behavioral Sciences, Unit of Neurophysiol., Univ. di Pavia, Pavia, Italy

Abstract: The existence of a functional interconnection between the cerebellum and neo-cortical areas subserving cognitive functions, such as the medial prefrontal cortex (mPFC), strongly stands for a cerebellar role in cognition. Alterations in cerebello-prefrontal cortex (mPFC) connections characterize several cognitive dysfunctions (such as schizophrenia), suggesting that the cerebellum might have a crucial impact on proper mPFC functioning. Cerebellar activity might impact on mPFC neuronal activity through two different projection pathways: the dopaminergic one, through the ventral tegmental area (VTA), and the glutamatergic one, through the mediodorsal and ventrolateral nuclei of the thalamus. Despite these findings, the impact of cerebellar activity on mPFC still remains to be elucidated. To get a deeper insight into the cerebello-mPFC connection we performed single-unit recordings *in vivo* from the prelimbic area (PrL) of mPFC in anesthetized mice and electrically stimulated the contralateral cerebellar dentate nucleus (DN). DN stimulation mainly elicited a pause in PrL background neuron firing, sometimes followed by an excitatory rebound. Furthermore, to investigate the nature of PrL responses, we used surface application of selective dopamine D1-like and D2-like receptor antagonists (SCH23390 and sulpiride, respectively) and of a $GABA_A$ receptor antagonist (gabazine). The D1-D2 receptor blockade modulated PrL neuron background discharge without affecting the pause usually observed in response to DCN stimulation. Conversely, this pause was suppressed by gabazine superfusion, suggesting a prominent role of local inhibition in controlling the phasic response of PrL neurons. Interestingly, the effect of $GABA_A$ receptor block on the pause was correlated with that of D1-D2 receptor block on background frequency. In summary, our data show that cerebellar activation silences PrL activity through local inhibitory circuits that remain to be identified. Possibly, cerebello-thalamic projections activate local cortical inhibitory interneurons. Moreover, PrL neuron background discharge is modulated by the dopaminergic system. Eventually, the interplay of the dopaminergic and GABAergic pathways regulates the signal-to-noise ratio in PrL neuron responses, revealing complexity in the cerebellar functional control of PrL.

Disclosures: D. Di Domenico: None. **I. Montagna:** None. **L. Moscato:** None. **S. Tritto:** None. **L. Mapelli:** None. **E. D'Angelo:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.07

Topic: B.06. Synaptic Transmission

Support: NINDS intramural research program (ZIA NS003168)

Title: In vivo plasticity between ventral hippocampal inputs and medial prefrontal cortex microcircuits in a mouse model of 22q11.2 deletion syndrome

Authors: T. T. CLARITY[1], R. M. MIKOFSKY[1], M. V. MYROSHNYCHENKO[1], K. GILCHRIST[1], *D. A. KUPFERSCHMIDT[1], J. A. GORDON[2,1];
[1]Integrative Neurosci. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD;
[2]Office of the Director, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Functional connectivity between rodent ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) supports cognitive functions such as spatial working memory (SWM), and is disrupted in *Df(16)A*$^{+/-}$ mice with a microdeletion syntenic to the schizophrenia-predisposing 22q11.2 microdeletion. Optogenetic inhibition of direct vHPC-mPFC neural projections or somatostatin-positive (SST+) interneurons in mPFC of wildtype (WT) mice impairs vHPC-mPFC connectivity and SWM performance, mimicking the effects of the microdeletion. To examine how functional interactions between these circuit elements may contribute to vHPC-mPFC dysconnectivity in *Df(16)A*$^{+/-}$ mice, and how these interactions may be altered to promote vHPC-mPFC connectivity, we tested the ability of vHPC-mPFC inputs to modulate *in vivo* activity of discrete mPFC neuronal populations in WT and *Df(16)A*$^{+/-}$ mice using an all-optical approach. ChrimsonR was expressed in vHPC neurons of adult male and female WT and *Df(16)A*$^{+/-}$ mice. GCaMP6f was expressed in mPFC SST+ or parvalbumin-positive (PV+) interneurons in separate cohorts (n=5-13). Red light pulses were delivered to the mPFC to excite vHPC terminals, and postsynaptic activation of GCaMP6f was monitored using fiber photometry. Stimulation-evoked photometric responses in SST+ interneurons varied systematically with pulse number (1-40) and frequency (1-40Hz), with maximal responses achieved by 40 pulses at 40Hz. SST+ interneuron responses in *Df(16)A*$^{+/-}$ mice were weaker than those in WT mice (p=0.03, main effect of Genotype, 3-way ANOVA). Repeated daily high-frequency stimulation over 12 days increased evoked SST+ interneuron responses for up to 50 days in both genotypes (p=0.006, Day x Stimulation, 3-way ANOVA). High-frequency stimulation in *Df(16)A*$^{+/-}$ mice potentiated SST+ interneuron responses to levels observed in WT mice without such stimulation. PV+ interneuron responses to high-frequency stimulation in WT mice were weak and biphasic; the negative component of this response was enhanced across stimulation days (p=0.03, main effect of Day, one-way ANOVA). These findings suggest that the capacity of vHPC inputs to engage mPFC SST+ interneurons may be impaired in *Df(16)A*$^{+/-}$ mice, and that repeated high-frequency stimulation of vHPC-mPFC inputs induces *in vivo* plasticity that may involve recruitment of SST+ interneurons. Future work will assess how this plasticity influences SWM performance. Together, these data reveal properties of cell-type-specific functional connectivity within intact vHPC-mPFC circuits that inform how discrete circuit elements may interact to mediate normal and disordered cognitive function.

Disclosures: T.T. Clarity: None. **R.M. Mikofsky:** None. **M.V. Myroshnychenko:** None. **K. Gilchrist:** None. **D.A. Kupferschmidt:** None. **J.A. Gordon:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.08

Topic: B.06. Synaptic Transmission

Support: CIHR: 142420

Title: Dynamic filtering of habenulo-raphe inputs by a serotonergic recurrent inhibitory network acting over behavioral timescales

Authors: *M. LYNN[1], S. D. GEDDES[1], M. CHAHROUR[1], S. MAILLÉ[1], E. HARKIN[1], E. HARVEY-GIRARD[1], S. HAJ-DAHMANE[3], R. NAUD[1], J.-C. BEIQUE[2];
[2]Cell. and Mol. Med., [1]Univ. of Ottawa, Ottawa, ON, Canada; [3]Univ. at Buffalo - The State Univ. of New York, Buffalo, NY

Abstract: The habenulo-raphe pathway is believed to be implicated in orchestrating optimal behavioral responses to aversive, threatening or stressful environments. Here, we consider how long-range inputs from lateral habenula (LHb) influence circuit dynamics in the dorsal raphe nucleus (DRN) using electrophysiological, computational and behavioral strategies. We find that habenulo-raphe afferents triggered classical monosynaptic excitation of 5-HT neurons, as well as strong disynaptic inhibition whose induction was steeply frequency-dependent and which persisted for seconds. This novel inhibition was mediated by a GIRK conductance activated by 5-HT1A receptors. To determine the network motifs underlying this protracted inhibition, we undertook optogenetic and pharmacological manipulations in DRN. These experiments revealed, unexpectedly, that 5-HT neurons are organized in a recurrent inhibitory network, refuting the classical model of autocrine activation of 5-HT1A autoreceptors. Electrical stimulation approaches revealed that these inhibitory connections exhibited robust, dramatic short-term facilitation that we formalized with a linear-nonlinear plasticity model. To examine how this nonlinearity affects input-output transformation, we constructed an experimentally-constrained network model of DRN which incorporated the experimentally-fitted plasticity model at inhibitory connections. We find that excitatory inputs led to paradoxical inhibition of DRN at high frequencies, and that this polarity switch was dependent on plasticity dynamics and not on recurrent inhibition itself. To test the physiological relevance of this computation for processing threat information from the LHb, we developed a simple auditory classical conditioning paradigm and tested key predictions of our model through *in vivo* optogenetics. We uncovered a nonlinear transformation where stimulating the habenulo-raphe pathway at high-frequency, but not at low frequency, *depressed* goal-directed anticipatory licking behavior. We suggest that the computation sustained by this circuit motif in the DRN categorizes synaptic inputs to implement optimal adaption of behavioural policies in threatening environments.

Disclosures: M. Lynn: None. **S.D. Geddes:** None. **M. Chahrour:** None. **S. Maillé:** None. **E. Harkin:** None. **E. Harvey-Girard:** None. **S. Haj-Dahmane:** None. **R. Naud:** None. **J. Beique:** None.

Poster

P103. Synaptic Transmission: Modulation by Neurotransmitters and Peptides II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P103.09

Topic: B.06. Synaptic Transmission

Support: NIH IRP

Title: Regiospecific knockout of PACAP expression and its impact on endocrine and behavioral consequences of restraint stress

Authors: *S. Z. JIANG[1], L. E. EIDEN[2];
[2]Sec Molec Neurosci, [1]NIMH/NIH, Bethesda, MD

Abstract: The neuropeptide PACAP is implicated in stress responding throughout the nervous system. PACAP in brain mediates both *behavioral* and *endocrine* responses to stress. PACAP deficiency has no effect on CORT elevation after 1-2 hr of restraint stress or even for 1 hr restraint stress repeated for 7 days, but does attenuate CORT elevation when stress occurs continuously for 2-3 hr of restraint stress repeated over several days. On the other hand, CRH mRNA and Fos elevation in PVN are PACAP-dependent even after just 1 hr of restraint (Jiang and Eiden, STRESS 19: 374, 2016). We deduce that PACAP is required not to *release* CRH, but rather to *sustain* the ability of CRH neurons to produce and secrete CRH by augmenting CRH mRNA production, thus allowing a higher rate of CRH peptide biosynthesis during stress. However, restraint-induced hypophagia even after 1 hr restraint is PACAP-dependent, indicating that this effect requires PACAP actions within circuits other than those controlling the HPA axis. In addition to activation of CRH PVN neurons, restraint stress also activates neurons of the centrolateral amygdala (CeL) and oval nucleus of BNST (ovBNST) that receive PACAPergic inputs from the extended lateral parabrachial nucleus (elPBn). PACAP terminals appear to synapse predominantly on PKCδ- and to some extent on CRH-expressing neurons of extended amygdala. Fos activation in ovBNST and CeL after 2 hr restraint is abolished in $PBn^{AAV\text{-}Cre}$::$PACAP^{fl/fl}$ mice, in which PACAP is ablated in nerve terminals of ovBNST and CeL, concommitant with attenuation of restraint-induced hypophagia. Fos activation in PVN, and CORT elevation after chronic restraint stress were maintained in $PBn^{AAV\text{-}Cre}$::$PACAP^{fl/fl}$ mice. Additional PACAPergic circuits participate in restraint stress responses. Sim1-Cre::$PACAP^{fl/fl}$ mice, in which PACAP expression throughout hypothalamus is ablated, showed attenuated hypophagic response to acute restraint stress. CamK2a-Cre:: $PACAP^{fl/fl}$ mice, in which PACAP expression is ablated in forebrain, but not in hypothalamus and brain stem, did not show

attenuation of restraint-induced hypophagia, but did exhibit deficits in restraint stress-induced HPA axis activation. Thus, Fos activation in PVN at 2 hr of acute restraint stress was attenuated in CamK2a-Cre::PACAP$^{fl/fl}$ mice, and this is reflected cumulatively in attenuated CORT elevation after chronic restraint stress (2 hr restraint stress repeated over 4 days). These results suggest that anatomically distinct PACAPergic circuits separately control endocrine and behavioral responses to stress.

Disclosures: S.Z. Jiang: A. Employment/Salary (full or part-time):; NIH IRP. **L.E. Eiden:** A. Employment/Salary (full or part-time):; NIH IRP.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.01

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

Support: ADDF-T-PEP-18-579974C
NIH/NIA-R01 AG059799

Title: The use of seeded models of tau propagation for evaluating nSMase2 inhibitors in Alzheimer's Disease

Authors: *M. M. MALVANKAR[1], B. S. SLUSHER[3], B. J. BELL[2], C. TALLON[1]; [2]Human Genet., [1]Johns Hopkins Sch. of Med., Baltimore, MD; [3]Neurol., Johns Hopkins Drug Discovery, Baltimore, MD

Abstract: Accumulation of amyloid-β (Aβ) peptide into plaques and aggregations of the microtubule associated binding protein Tau (MAPT) into neurofibrillary tangles (NFT) are key pathological hallmarks of Alzheimer's Disease (AD). Importantly, the degree of spread of phosphorylated tau is strongly correlated with AD's progressive neurodegeneration, as well as cognitive and functional impairments. Therefore, there is a high clinical need for novel therapeutics which aim to limit the propagation of tau and may stop or slow the progression of AD in patients. Recent evidence has highlighted the importance of extracellular vesicles (EVs) in enabling the transcellular transmission of pathological tau in the brain in a 'prion-like' manner and identified the inhibition of EV biogenesis via small-molecule inhibitors of nSMase2 as a potential avenue for clinical intervention. However, drug discovery efforts have been hampered by the use of transgenic mouse models which express human Tau in the majority of neurons, making it difficult to study the propagation of phosphorylated tau from neuron to neuron along defined pathways as occurs in AD patients. To better assess the preclinical efficacy of nSMase2 inhibitors, our lab has operationalized a rapid tau-seeded model of tau propagation, in which the gene for mutant human tau is packaged in an AAV vector and stereotaxically injected

unilaterally into the mouse hippocampal CA1 region. The mice are then treated with test compounds or control for 6 weeks, at which time the mice are sacrificed, and the spread of tau and its phosphorylated species is quantified via histology in the contralateral dentate gyrus (DG). We have demonstrated that both total tau and the phosphorylated tau species pThr181 are consistently observed in the neurons of the granule cell layer of the ipsilateral DG, and spread to neurons and $GFAP^+$ astrocytes in the contralateral DG hilus. Histological examination allows for quality control of the injected mice, and propagation quantification is performed by measuring the contralateral/ipsilateral ratio of mean fluorescence intensity and positive cell count for phosphorylated tau species. Preliminary treatment studies utilizing the potent nSMase2 inhibitor 1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b] pyridazin-8-yl) pyrrolidin-3-yl) carbamate (PDDC) have confirmed that the inhibition of nSMase2 significantly reduces the tau spread between hemispheric hippocampi. These results demonstrate the unique value of this rapid propagation AD mouse model for drug discovery efforts focused on attenuating the propagation of pathological tau *in vivo*.

Disclosures: M.M. Malvankar: None. **B.S. Slusher:** None. **B.J. Bell:** None. **C. Tallon:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.02

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

Support: NIH Grant AG051085
Owens Family Foundation
Cure Alzheimer's Fund
NIH Training Grant T32GM008715

Title: Extracellular Tau Oligomers Disrupt the Axon Initial Segment by a Mechanism Dependent on Intracellular Tau

Authors: *M. N. BEST, K. H. SILLER, Y. LIM, N. KIM, N. N. FERENC, D. BIGLER WANG, J. W. MANDELL, G. S. BLOOM;
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Abstract: BACKGROUND. Mislocalization of tau, a protein normally enriched in axons, into the somatodendritic compartment is an early event in Alzheimer’s disease (AD) and non-AD tauopathies, and can be caused by extracellular tau oligomers (tauOs) (Swanson, et al. 2017. J Alz Dis 58: 803-820). Recent studies focusing on the role of the axon initial segment (AIS) as a diffusion barrier between the somatodendritic compartment and the axon point towards a mechanistic role for AIS disruption in tau mislocalization. We, therefore, sought to determine if

extracellular tauOs adversely affect AIS structure.
METHODS. Tau oligomers were prepared using purified human recombinant 2N4R tau, which aggregated in the presence of the polyunsaturated fatty acid, arachidonic acid. Quantitative immunofluorescence microscopy of cultured neurons for the AIS structural proteins, ankyrin G, βIV-spectrin, and TRIM46, was used to analyze AIS morphology in primary cortical neurons derived from wild type and tau knockout (KO) primary cortical mouse neurons. Following ~18-hour incubations with tauOs or vehicle, cells were labeled with antibodies to tau, the somatodendritic marker, MAP2 and the three AIS structural proteins. Blinded imaging and data analysis were completed to assess immunofluorescence intensity, AIS length, and distance of the AIS from the soma.
RESULTS. TauOs caused substantial loss of AIS structural proteins and shortening of the AIS in wild type mouse neurons, but not in tau KO neurons. Lentiviral expression of human 2N4R tau in tau KO neurons rescued these effects of tauOs on the AIS.
CONCLUSIONS. Endogenous cytoplasmic tau is not required for AIS formation, but extracellular tauOs cause a major loss of structural proteins along the entire length of the AIS, coupled with AIS shortening from its distal end. Remarkably, this AIS disruption caused by extracellular tauOs requires intracellular tau. These collective results raise the possibility that tau mislocalization in AD and non-AD tauopathy neurons is a consequence of AIS disruption by extracellular tauOs.

Disclosures: M.N. Best: None. **K.H. Siller:** None. **Y. Lim:** None. **N. Kim:** None. **N.N. Ferenc:** None. **D. Bigler Wang:** None. **J.W. Mandell:** None. **G.S. Bloom:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.03

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

Support: L.I.F.E. FOUNDATION Research Grant
Cincinnati Children’s Mind Brain Behavior Research Innovation/Pilot Fund

Title: A mechanism for DEK loss and its association with Alzheimer's disease

Authors: *A. GREENE[1], M. B. SOLOMON[1], L. PRIVETTE VINNEDGE[2];
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Abstract: DEK, a chromatin remodeling, nuclear protein, has been linked with neurodegenerative disorders such as Alzheimer’s disease (AD) (Ghisays et al., 2018), Huntington’s disease (Christodoulou et al., 2020), and age-related dementias (Ghisays et al., 2018, O’Donovan et al., 2018). In the periphery, DEK has cellular functions in DNA replication

and repair, cell proliferation, and apoptosis inhibition. Recently, we reported that DEK expression is necessary for proper neurite formation and that its deficiency leads to cell death and both Tau overexpression and hyperphosphorylation (Greene et al., 2020). The purpose of the current study is to elucidate the mechanism by which DEK loss leads to Tau hyperphosphorylation, with the goal of illuminating novel pathways of Tauopathy in Alzheimer's disease. To identify a functional role for DEK in AD-related phenotypes, SH-SY5Y cells were transduced with DEK-targeting lentivirus (DEKsh) or non-targeting lentivirus (NTsh) and subsequently differentiated into a neuronal-like phenotype. One week before the end of the differentiation process, cells were treated with kinase inhibitors for CamKII, ERK, PKC, PKA, DYRK, CDK5, or GSK3β; these kinases are associated with Tau pathology in Alzheimer's disease. At the end of differentiation, the relative expression levels of phosphorylated Tau and kinase activation markers were determined via immunofluorescence and western blotting. We found that the inhibitors of ERK, DYRK, CDK5, and GSK3β reduced phosphorylated Tau (Ser262) to control levels, while treatment with other kinase inhibitors did not rescue the Tau pathology phenotype in the DEK-deficient (DEKsh) cells. We observed increased activation of these same kinases in DEKsh cells. Previous reports showed that DEK overexpression leads to decreased extracellular glutamate, and that CDK5 can be activated upon the presence of excess extracellular glutamate. Therefore, we used a glutamate bioluminescent assay and found that DEKsh cells showed elevated levels of extracellular glutamate. Our results indicate that DEK loss leads to increased glutamate secretion from neuronal cells, which may have downstream consequences such as the observed activated CDK5 and cell death. Further potential downstream effects include ERK and GSK3β activation, contributing to hyperphosphorylated Tau after DEK loss in this cell model. These data may inform us of the molecular mechanism(s) by which DEK loss leads to cellular and molecular phenotypes associated with Alzheimer's disease or other age-related dementias.

Disclosures: A. Greene: None. **M.B. Solomon:** None. **L. Privette Vinnedge:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.04

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

Support: R01AG044372
R01NS082730
Secchia Family Foundation Research Fund

Title: Assessing pathogenic conformations of tau in primary neurons seeded with human-derived pathological tau

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Abstract: One of the pathological hallmarks of Alzheimer's disease (AD) is the accumulation of pathological tau inclusions, and the multimerization of tau is likely a critical part of tau's role in disease pathogenesis. Tau is capable of seeding aggregation in cells; however, the extent to which specific disease-associated pathogenic conformations can be effectively seeded remains an area of active investigation. To help address this gap, we seeded aggregation in primary neurons and assessed known pathogenic tau conformations that are linked to mechanisms of tau-induced toxicity. We used hippocampal primary neurons derived from tau knock-in mice that express all six human tau isoforms. We seeded aggregation by treating neurons with human AD brain-derived tau (AD-tau). The AD-tau samples were characterized by transmission electron microscopy (TEM) and biochemical assays that measure total tau and pathological modifications, such as phosphorylation (e.g. PHF-1+ tau) and two conformation specific antibodies (TNT1 and TOC1). TNT1 labels conformation-dependent display of an N-terminal domain known as the phosphatase activating domain (PAD) and TOC1 labels oligomeric tau, both of which are linked to toxicity. The AD-derived material showed morphologies consistent with small pieces of the characteristic paired helical filaments of AD on TEM, phosphorylation at the PHF-1 site on western blot, and the presence of oligomeric tau and PAD-exposed tau species in sandwich ELISAs. Following treatment of neurons with AD-tau, we used immunostaining to label TNT1 and TOC1 conformations at multiple time points. We observed a robust increase in TNT1 labeling over time in AD-tau-treated primary neurons, but little to no evidence of TOC1+ oligomeric tau species. To begin assessing consequences of tau seeding in this model, we determined whether overt cell loss occurred up to 28 days after seeding. There were no significant effects on the number of neurons or astrocytes in seeded cultures suggesting that robust degeneration did not occur. Ongoing studies will assess additional functional consequences (e.g. axonal transport impairment) and potentially earlier measures of toxicity (e.g. synaptic and axonal toxicity) in seeded neurons. Together, our results suggest that seeding primary neurons with AD-tau drives some of the pathogenic conformations observed in human disease, but overt degeneration does not occur within 28 days of seeding. The presence of pathological forms of tau but lack of overt degeneration could indicate that more time is needed and/or that the functional consequences of these tau species are more subtle leading to synaptic loss or axonal degeneration.

Disclosures: R.L. Mueller: None. **N.M. Kanaan:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.05

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

Support: Rainwater Charitable Foundation

Title: Tau^{Agg}: a luminescence-based in vivo system to detect tau protein aggregation in drosophila

Authors: *S. A. LEVY, E. M. GONZALES, B. FROST;
Cell Systems and Anat., UT Hlth. San Antonio, San Antonio, TX

Abstract: Tauopathies are characterized by fibrillar tau protein aggregates. In Alzheimer's disease, the most common tauopathy, these tau aggregates form neurofibrillary tangles, one of the major pathological hallmarks of the disease. Strikingly, tau pathology propagates through the brain hierarchically over time and appears to spread along circuitry, suggestive of a prion-like mechanism. In recent years, tau has been shown to spread between cells *in vitro* and *in vivo* and to recruit soluble, non-pathogenic tau into new aggregates. However, the mechanisms mediating and regulating these processes have not been well characterized. We have developed a *Drosophila* model of tau protein aggregation, termed tau^{AGG}, to provide a tool to study the processes underlying it in a short-lived, cost-effective animal model with a large genetic toolkit. We found that the P301L mutant of full-length human tau (tau^{P301L}) forms oligomers in *Drosophila* neurons, suggesting a capacity to seed aggregation. Based on this, we created the tau^{AGG} system, where tau^{P301L} fused to either the C-terminal or N-terminal portion of luciferase is expressed pan-neuronally. In this split-luciferase assay, the close proximity of C-terminal and N-terminal luciferase fragments caused by aggregation will produce luminescence and allow us to quantify tau-tau interaction *in vivo* in a screenable manner. We found that tau^{AGG} animals exhibit significantly higher luminescence signal than non-transgenic control animals and animals expressing a control construct, where EGFP is fused to the split-luciferase components, indicating that tau^{AGG} selectively quantifies tau-tau interaction. A *Drosophila* model of tau aggregation allows us to investigate the mechanisms controlling this phenomenon in adult, aging brains. The genetic tools available to *Drosophila* research, as well as straightforward delivery of drugs through *Drosophila* food make it possible to screen genetic and pharmacological modifiers of tau aggregation with the long-term goal of developing treatment strategies targeting the prion-like spread and seeding of pathogenic tau. In this context, a luminescence-based model of tau seeding is well-suited for screening approaches to interfere with putative mechanisms, since luminescence can be acquired in living animals, making longitudinal studies feasible.

Disclosures: S.A. Levy: None. **E.M. Gonzales:** None. **B. Frost:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.06

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

Support: NIH Grant R25GM131956

Title: Investigating the effectiveness of galantamine hydrobromide in reducing symptoms of Alzheimer's disease in Drosophila melanogaster

Authors: *E. R. NIEMAN, S. L. FOX, H. B. SHAH, D. D. LENT;
Biol., California State University, Fresno, Clovis, CA

Abstract: Impaired sleep schedules, difficulty with spatial learning and memory, and reduced ability to plan activities have all been noted symptoms of patients with Alzheimer's disease (AD). AD pathology is characterized by an accumulation of neurofibrillary tangles, tau aggregates, and beta-amyloid plaques. One common characteristic of patients with AD is a reduction of acetylcholine (ACh), a neurotransmitter associated with memory and learning processing, which is broken down in the synapse by the enzyme acetylcholinesterase (AChE). Our research focused on analyzing and quantifying changes in behavioral patterns in flies expressing human tau in the mushroom bodies and ellipsoid body, brain regions that have a role in learning and memory and spatial cognition, that have been treated with an AChE inhibitor. We hypothesized that, similar to humans and vertebrate models, inhibiting AChE would slow the progression of the disease and help improve behavioral function in the fruit fly model. Fruit fly lines were created to express tau in either the mushroom bodies or the ellipsoid body. The AChE inhibitor galantamine hydrobromide was used as a treatment and its treatment effect on the progression of early tau pathologies was studied. Flies expressing tau from 1-7 days old were treated and compared to untreated and control flies in three assays. To assess sleep, the Drosophila Activity Monitor was used to monitor the fly's day-night activity and circadian rhythms. To assess visual place learning, flies were trained to locate a hidden cool spot on a hot surface using visual cues and were then tested for memory of that location. To assess visuo-motor planning, flies were analyzed in a tap-down apparatus that required them to negotiate a high-contrast visual barrier. The results from the behavioral assays revealed quantifiable differences in behavior between control flies, diseased flies, and flies receiving the galantamine hydrobromide treatment. In the sleep study, the flies of different lines and experimental conditions had differing levels of activity and sleep patterns. In the visual place learning experiment, treatment had a demonstrable effect on diseased flies' ability to associate a cool spot with patterns on walls of the thermal maze assay. Finally, in the visuo-motor planning study, the flies' efficiency in navigating the assay as a function of time varied considerably between the three groups but results showed an impact of treatment on performance. Ongoing experiments suggest that drug treatment may rescue diseased phenotypes and bring them closer to the controls. This helps advance the fruit fly model as a viable method for studying AD.

Disclosures: E.R. Nieman: None. **S.L. Fox:** None. **H.B. Shah:** None. **D.D. Lent:** None.

Poster

P224. Tau: Animal and Cellular Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P224.07

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

Support: NIH Grant 5U01AG061357-03

Title: Functional validation of the cytoskeletal protein Plectin in Alzheimer's disease

Authors: *T. V. LEE[1], K. ALLISON[2], N. T. SEYFRIED[3], A. I. LEVEY[4], J. M. SHULMAN[2]; [1]Neurol., [2]Baylor Col. of Med., Houston, TX; [3]Human Genet., Emory Sch. Med., Atlanta, GA; [4]Dept Neurol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia. The genetic causes for the majority of AD cases and the underlying biology of pathogenesis remain unknown. Recent large scale proteomics studies on AD patient brains have identified multiple novel protein networks and targets that are highly correlated with AD diagnosis and neuropathology. One of the most significantly AD-correlated protein networks identified in this analysis contains the cytoskeletal protein Plectin as a protein network hub. Plectin functions to crosslink different components of the cytoskeleton and plays a role in axonal transport of synaptic proteins. To validate Plectin as potential AD target of analysis, we performed functional studies in the animal model, *Drosophila melanogaster* and human neuronal cell culture. Expression analysis in *Drosophila* exhibit ubiquitous Plectin expression within the adult brain with strong enrichment in neuronal rich regions. Using *Drosophila* models of AD overexpressing human Tau, altering neuronal levels of Plectin enhances age dependent Tau-induced neurotoxicity and locomotor dysfunction. In addition, Plectin modulates Tau-phosphorylation and aggregation in both *Drosophila* brains and human neurons. Moreover, in the absence of Tau overexpression, decreasing neuronal Plectin levels result in abnormal neuronal transmission as measured by the electroretinogram, confirming Plectin's role in neuronal function. Taken together this data identifies and functionally validates Plectin as a regulator of Tau-induced neurodegeneration and identifies Plectin as regulator of Tau dynamics and neuronal function. Continued mechanistic analysis in both *Drosophila* and human neuronal cultures with determine Plectin's role in AD neuropathogenesis and elucidate the underlying role of the cytoskeleton in Tau-induced neurodegeneration.

Disclosures: T.V. Lee: None. **K. Allison:** None. **N.T. Seyfried:** None. **A.I. Levey:** None. **J.M. Shulman:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.01

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

Support: NIH Grant AG029777
NIH Grant AG066384
NIH Grant AG062021
NIH Grant AG057325
NIH Grant AG053150
NIH Grant AG033474
ADDF Grant NS49442

Title: Reduction of tau to baseline levels and rescue of motor impairment in a therapeutic study in aged JNPL3 mice treated with small molecule OLX-07010 targeting tau self-association

Authors: *J. MOE[1], P. LOPEZ[2], D. PATEL[2], H. JIMENEZ-BRAVAR[3], L. ADRIEN[3], A. WOLIN[3], J. KOPPEL[3], P. DAVIES[3], *E. DAVIDOWITZ[1];
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Abstract: Tau has been implicated in the pathogenesis of multiple neurodegenerative diseases associated with the accumulation of abnormal species of tau, collectively called tauopathies. Mutations in MAPT can cause a range of rare inherited tauopathies to develop due to overproduction of specific tau isoforms or changes in the structure of tau, both of which can cause tau to aggregate. In AD, tau pathology is driven by numerous posttranslational modifications that lead to loss of normal function and/or gain of toxic function. Neurofibrillary tangles, composed primarily of tau, accumulate in a highly reproducible spatiotemporal order demonstrating a close association between tau aggregation and AD progression. Tau oligomers have neurotoxic effects on the genome, mitochondria, cell signaling, synaptic plasticity, microtubule assembly, the cytoskeleton and protein clearance mechanisms. Oligomerix has shown that tau oligomers cause disruption of neuronal signaling and inhibit the formation of memory in mice (Fá, M. et al., 2016). We have found that certain forms of tau oligomers are toxic when applied to cultured neurons, whereas tau monomer was not toxic at the same concentrations (Tian, H. et al., 2013). This program seeks to discover and develop disease modifying therapeutics for AD and related dementias using a small molecule approach targeting tau self-association to impact the accumulation of all tau aggregates. The in vivo efficacy of our proprietary small molecule OLX-07010 in this therapeutic study builds upon results from the preventive studies in the htau (Davidowitz, E. et al., 2020) and tau P301L JNPL3 mouse models of tauopathy. This blinded study was independently performed using homozygous female JNPL3 mice that were aged to 7 months and treated for 5 months with compound administered in feed for daily doses of 40 and 80 mg compound per kg mouse. A vehicle feed control group and

baseline cohort (7 months-of-age) were also included in the study. Treatment with OLX-07010 caused a decrease in the accumulation of heat stable, self-associated, Sarkosyl-insoluble tau aggregates, and phosphorylated tau with statistical significance to baseline levels. Moreover, the reduction in tau aggregation in the cortex and its aberrant phosphorylation correlated with improved motor performance in the Rotarod assay and in open field arena analyses. Preclinical studies are being performed for an IND submission to the FDA to enable planned first-in-human studies.

Disclosures: **J. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **D. Patel:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc.. **H. Jimenez-Bravar:** None. **L. Adrien:** None. **A. Wolin:** None. **J. Koppel:** None. **P. Davies:** None. **E. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc..

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.02

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

Support: MICIU-Biomedicina (RTI2018-098885-B-100

Title: CB1 receptor activation as a neuroprotective strategy in TDP-43 dependent frontotemporal dementia

Authors: L. GARCÍA TOSCANO[1,2,3], I. SANTOS-GARCÍA[1,2,3], C. RODRÍGUEZ-CUETO[1,2,3], J. FERNÁNDEZ-RUIZ[1,2,3], ***E. DE LAGO FEMIA**[1,2,3];
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Abstract: TAR DNA-binding protein 43 (TDP-43) is involved in the regulation of RNA stability, transport, and splicing. Its dysregulation has been linked with some forms of frontotemporal dementia (FTD). The therapeutic outcome in FTD is currently limited. Our group

has recently initiated studies aimed at exploring cannabinoid-based therapies as a promising option for the treatment of FTD, given their relevant neuroprotective and anti-inflammatory profile demonstrated in other neurological disorders. For this objective, we have used mice that overexpress TDP-43 in forebrain neurons under the control of α-CaMKII promoter, which recapitulate important neuropathological signs of FTD (provided by Shen; Tsai et al., J Exp Med 2010). The analysis of the endocannabinoid system in these mice in a preliminary study proved adaptive changes in some elements of this system (e.g., increase in the anandamide-synthesizing enzyme NAPE-PLD, and reduction in the hydrolyzing enzyme FAAH) in some key CNS structures in FTD (e.g., medial prefrontal cortex, hippocampus), which may be interpreted as an endogenous protective response aimed at elevating anandamide levels in these CNS structures. Given these results, our follow up studies have tried first to inactivate FAAH enzyme using the selective inhibitor URB597 administered i.p. at the dose of 0.2 mg/kg on alternate days from pre-symptomatic phases (PND45) to symptomatic stages (PND90). Our data proved that URB597 delayed the appearance of FTD signs (e.g. cognitive deterioration measured in the NOR test), whereas preserved pyramidal neurons in the medial prefrontal cortex and in the hippocampal CA1 layer (measured with Ctip2 and NeuN immunostaining, respectively), and attenuated the elevated glial immunoreactivity (measured with the marker of reactive astrocytes, S100-β, in the medial prefrontal cortex, and with the marker of microglia, Iba-1, in the CA1 layer). To explore whether these effects may involve the activation of the CB_1 receptor, we conducted a second experiment by treating FTD mice with the selective CB_1 receptor agonist ACEA administered i.p. at the dose of 1.5 mg/kg daily during the same time range. The treatment with ACEA also delayed the appearance of FTD signs and these effects were eliminated by co-administration of ACEA with the selective CB_1 receptor antagonist rimonabant. In summary, our data confirm the potential of modulating the endocannabinoid system as a therapy against TDP-43-induced neuropathology in FTD, in particular by activating the CB_1 receptor which serves to preserve neuronal integrity and to limit glial reactivity, resulting in an improvement of cognitive deficits.

Disclosures: L. García Toscano: None. **I. Santos-García:** None. **C. Rodríguez-Cueto:** None. **J. Fernández-Ruiz:** None. **E. de Lago Femia:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.03

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

Title: Cola nitida ameliorate cardiometabolic dysfunction and amnesia induced by dexamethasone and scopolamine in male mice

Authors: *L. ADEDAYO[1], O. MICHAEL[2], F. AWOBAJO[5], O. OLUMOROTI[3], O. BAMIDELE[3], N. AITOKHUEHI[6], O. MORAKINYO[4], S. ONASANWO[7];
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Abstract: Abstract Accumulative evidence has indicated that vascular risk factors including cardiometabolic disorders have been shown to be predictors of amnesia. *Cola nitida* is natural nuts with numerous bioactive compounds that have shown tremendous therapeutic potentials such as antioxidants, anti-nociception and anti-inflammatory properties. The precise reason why cardiovascular and cardiometabolic conditions are risk factors for dementia remains unknown. However, this study investigated the therapeutic potentials of the methanol extract of *Cola nitida* on cardio-metabolic dysfunction and amnesia induced by dexamethasone and scopolamine in mice. Male mice aged 14 weeks were given (p.o.) vehicle, (3mg/kg i.p) scopolamine (SCO), 0.2 mg/kg dexamethasone (DEX) plus 3mg/kg SCO, 50mg/kg *Cola nitida* (CN) plus 3mg/kg SCO, 0.2mg/kg DEX plus 3mg/kg SCO plus 50mg/kg CN, 0.2 mg/kg DEX plus 3mg/kg SCO plus (1mg/kg p.o.) donepezil daily for 7 days (n=5 per group) using Morris water maze (MWM) and Y-maze tests to assessed memory impairment. Glucose dysmetabolism was assessed by elevated fasting blood glucose, impaired glucose tolerance test. Treatment with CN led to significant increase in time spent in the quadrant in the probe trial (MWM) and increase in percentage alternation (Y-maze) tasks. On the other hand, CN led to decrease in escape latency time in MWM. Also, *Cola nitida* significantly decrease the acetylcholinesterase activity in the brain, Malondialdehyde, lactate dehydrogenase, triglycerides and glucose levels, however, CN improved glutathione reductase, reduced glutathione in the heart and brain homogenates. This result demonstrates that *Cola nitida* enhances memory through neuro-behavioural, acetylcholinesterase and antioxidant activities. On the other hand, CN ameliorate cardiometabolic dysfunction by antioxidant activity, decrease triglyceride accumulation and reduced glucose level in the heart. Therefore, *Cola nitida* would impact positively on cardiometabolic disorders and memory impairment.Keywords: Cola nitida, cardiometabolic dysfunction, amnesia, scopolamine, dexamethasoneWord count:284

Disclosures: L. Adedayo: None. **O. Michael:** None. **F. Awobajo:** None. **O. Olumoroti:** None. **O. Bamidele:** None. **N. Aitokhuehi:** None. **O. Morakinyo:** None. **S. Onasanwo:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.04

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

Support: NIH R21AG069140-01

Title: A branched-chain amino acid (BCAA)-lowering compound reverses metabolic dysregulation and enhances cortical insulin signaling in a mouse model of Alzheimer's disease

Authors: *C. A. MULLINS, H. SHAH, R. B. GANNABAN, M. B. SIDDIK, A. C. SHIN; Texas Tech. Univ., Lubbock, TX

Abstract: Metabolic dysregulation including abnormal glycemic control and insulin resistance is associated with age-related cognitive decline, thus these markers may be a promising target for prevention and treatment of neurodegenerative diseases such as Alzheimer's disease (AD). Studies show that BCAAs can induce insulin resistance and impair glucose metabolism. Based on our recent findings that plasma BCAAs are elevated in AD mice and humans compared to healthy subjects, we hypothesized that lowering BCAAs will correct metabolic impairments leading to alleviation of AD-related brain pathology and cognitive deficits. To this end, we treated 2-month-old C57BL/6J SJL wild-type (WT) and 5xFAD mice with either vehicle or 3,6-dichlorobenzo[b]thiophene-2-carboxylic acid (BT2), a small molecule that lowers plasma BCAAs by indirectly activating branched-chain α-keto acid dehydrogenase (BCKDH), the rate-limiting enzyme for BCAA degradation. Animals were separated into either WT vehicle (n=6), WT BT2 (n=6), 5xFAD vehicle (n=4), or 5xFAD BT2 (n=4) group. Blood was collected at baseline and 30 days after daily intraperitoneal injection of BT2 or vehicle to measure blood glucose and plasma insulin. Y-maze and novel object recognition test were also conducted to assess cognitive function. Insulin was injected systemically before sacrifice to assess insulin signaling in the brain.

We confirmed the stimulating effect of BT2 on BCAA breakdown in the liver by observing a reduction in phosphorylated BCKDH, the inactive form of the enzyme. Our results show that BT2 significantly lowered plasma insulin in 5xFAD mice compared to baseline. Likewise, 5xFAD mice had significantly higher blood glucose compared to WTs at baseline, but BT2 was able to completely normalize glucose. While there was no change in Abeta-42 in the cortex or cognitive function between groups, 5xFAD mice displayed enhanced insulin-stimulated insulin signaling following BT2 treatment.

Our findings suggest that BT2 is a novel therapeutic compound that may correct metabolic dysregulation and brain insulin resistance which may in turn slow AD progression. We are currently investigating potential effects of BT2 on neuronal health, mitochondrial and oxidative stress, inflammation, and neurotransmitter synthesis in AD mice. We did not observe any effects of BT2 on cognitive function most likely due to the early age of 5xFAD mice employed here. We plan to repeat this study with older mice in future studies.

Disclosures: C.A. Mullins: None. **H. Shah:** None. **R.B. Gannaban:** None. **M.B. Siddik:** None. **A.C. Shin:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.05

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

Support: AHA CDA 856826
AHA 19CDA34660032
NIH 1R01NS096225-01A1
AHA 19TPA34850047

Title: The role of long-chain acyl-CoA synthetases 3 (ACSL3) in age-induced vascular dementia

Authors: C. CITADIN[1], G. CLEMONS[1], C. ACOSTA[1], M. S. UDO[2], W. CARR[2], R. H.-C. LEE[2], H. LIN[2], ***C. Y.-C. WU**[2];
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Abstract: Alzheimer's disease (AD) is one of the most common neurodegenerative diseases in the United States. Risk factors for AD are associated with age and gender. Women are more likely to develop a rapid progression of dementia than men with a greater risk of developing vascular dementia as compared to males. Cerebral blood flow derangements found in AD are thought to be the major cause of brain dysfunction and neurological deficits. AD-mediated hypoperfusion plays a vital role in vascular dementia-related neuroinflammation, mitochondrial dysfunction, neuronal cell death, and neurological deficits. Therefore, the major challenge is to alleviate the development and progression of vascular dementia. We previously discovered that mRNA and protein expression of long-chain acyl-CoA synthetase 3 (ACSL3) in brain regions were significantly decreased with the aged AD mice. Specific agonist of ACSL3 alleviated AD-mediated hypoperfusion, neuroinflammation, and mitochondria dysfunction, with enhanced neuronal survival and improved functional learning/memory. Our preliminary data suggest that decreased ACSL3 is detrimental in an aged 3xTg-AD mouse model (enhanced β-amyloid and tau aggregation and accumulation). Our central hypothesis is that ACSL3 is critical for age-related brain function to maintain cerebral blood flow and the mitochondria, and prevent neuroinflammation and learn/memory degradation. We propose to explore the pathophysiological role of ACSL3 in the aged brain. The present study can lead to novel therapies/targets against AD brain progression by investigating the pathophysiological role of ACSL3 in cerebral blood flow regulation and neuroprotection.

Disclosures: C. Citadin: None. **G. Clemons:** None. **C. Acosta:** None. **M.S. Udo:** None. **W. Carr:** None. **R.H. Lee:** None. **H. Lin:** None. **C.Y. Wu:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.06

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

Support: Retina France
Association Syndrome de Wolfram
Fondation pour l'Audition

Title: Phenotypic characterization of a novel zebrafish model of Wolfram syndrome, a rare genetic disease

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Abstract: Wolfram syndrome (WS) is a rare neurodegenerative disease associating deafness, optic atrophy, diabetes and neurological disorders. Currently, no treatment is available for patients and only medical devices can improve their quality of life. The mutated gene, *WFS1*, encodes an endoplasmic reticulum (ER)-resident glycoprotein, Wolframin. We previously demonstrated that Wolframin regulated Ca^{2+} transfer from the ER to the mitochondria, thus modulating mitochondrial function. In WS condition, WFS1 is not functional and can no longer ensure its essential cellular functions, leading to cell death in different tissues. Zebrafish, which are vertebrate models of disease, have become established tools for drug discovery throughout the organism. The zebrafish genome is duplicated with two, *wfs1a* and *wfs1b*, genes. We developed a zebrafish mutant line by ENU mutagenesis, *wfs1ab*^{KO}, with mutation in both genes to completely invalidate Wolframin's function. The *wfs1ab*^{KO} line was fully characterized at these behavioral and cellular levels. We investigated vision, hearing, locomotion, morphological markers, ER stress factors, glycaemia and mitochondrial function. The *wfs1ab*^{KO} line showed an increased locomotion in visual motor response and in touch escape response, showing visual deficit and/or exacerbated anxiety. Acoustic startle response was unchanged thus suggesting an absence of hearing loss. ER stress response markers were analyzed by qPCR and Western blot. The absence of Wfs1 decreased the expression of *eif2s1* and *chop* in physiological condition but also impacted Tunicamycin-induced ER stress response, as revealed by the interaction between the genotype and tunicamycin treatment for *atf6* and *ire1* genes. Metabolic analysis using the Seahorse analyzer showed an increase in basal and maximal mitochondrial respiration and in ATP production in *wfs1ab*^{KO} larvae compared to controls. Taken together, these data validated the *wfs1ab*^{KO} zebrafish line as a model of WS and confirmed the impact of WFS1 on ER stress and mitochondrial pathways. Our ultimate goal is to use the *wfs1ab*^{KO} line as an efficient screening model to identify small active molecules able to correct or block WS symptoms.

Disclosures: L. Crouzier: None. **C. Diez:** None. **E.M. Richard:** None. **M. Denus:** None. **T. Maurice:** None. **B. Delprat:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.07

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

Support: NIH Grants RF1-AG044342, RF1-AG070296, R21-AG065693, R01-NS092918, R01-AG062135 and R56-NS113549
NIH Grants AG062135, NS108686, NS086074, NS092093
NIH Grants P30-AG10161, R01-AG17917
start-up funds from the University of Minnesota Foundation and bridge funds from the Institute of Translational Neuroscience
Training grant support for graduate students T32-NS105604
We are indebted to the Strom and Moe families for their gift.
We thank the study participants and staff of the Rush Alzheimer's Disease Center.

Title: Sex-dependent effects of alpha-synuclein silencing in a mouse model of Alzheimer's disease

Authors: *J. L. BROWN[1], G. BOYLE[2], D. HART[2], T. BROWN[3], M. LACROIX[2], M. KIM[2], M. A. SHERMAN[2], M. SUNG[2], T. COLE[4], J. A. SCHNEIDER[5], M. K. LEE[2], D. A. BENNET[5], H. B. KORDASIEWICZ[4], S. E. LESNE[2];
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Abstract: *Objectives*: Evidence is accumulating that Alzheimer's disease (AD) and Parkinson's disease, two of the most common neurodegenerative disorders, might share signaling pathways that could account for common pathology and symptomology. We previously described how genetically ablating *SNCA* encoding alpha-synuclein (αSyn) in a mouse model of AD improved survival and spatial memory. Antisense oligonucleotides (ASOs) are a clinically tested method of transiently lowering target gene transcripts. We investigated whether treatment with an ASO targeting murine *SNCA* mRNA improved cognition in mice overexpressing human amyloid precursor protein. *Methods*: Five-month-old male and female mice were injected with ASO^{SNCA} and learning and memory were tested at 6 months using the Barnes circular maze. *Results*: The murine ASO^{SNCA} was successful at lowering both *SNCA* mRNA and protein, and improving cognition in male mice. Female mice, however, displayed impaired memory retention. In addition, constitutive ablation of *SNCA* in female mice also worsened spatial memory. Since mediators of *SNCA* and its abundance might impact responses to ASO^{SNCA} treatment, we used

human samples from the Religious Orders Study to investigate potential sex differences in αSyn protein and its modulators. While confirming that soluble αSyn was increased in male and female AD brains, we also observed sex-specific differences in beta-synuclein (βSyn), an endogenous regulator of αSyn. βSyn was decreased in female AD brains but not in male brains, and cognitive scores for women in the AD group were inversely correlated with βSyn abundance. *Conclusions*: These results highlight a novel sex difference in our understanding of AD pathogenesis and may have profound implications for future translational studies.

Disclosures: J.L. Brown: None. **G. Boyle:** None. **D. Hart:** None. **T. Brown:** None. **M. LaCroix:** None. **M. Kim:** None. **M.A. Sherman:** None. **M. Sung:** None. **T. Cole:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Provided drug (alpha-synuclein ASO). **J.A. Schneider:** None. **M.K. Lee:** None. **D.A. Bennet:** None. **H.B. Kordasiewicz:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Provided drug (alpha-synuclein ASO). **S.E. Lesne:** F. Consulting Fees (e.g., advisory boards); Scientific consultant for Acelot, Inc. (no relation to the current work).

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.08

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

Support: NIH Grant R56AG05755501

Title: The effects of the antidepressant drug agomelatine on the Tg-F344-AD transgenic rat model of Alzheimer's disease

Authors: *G. TERRY[1,4], L. XIE[2], P. ROCKWELL[1], P. SERRANO[3], M. FIGUEIREDO-PEREIRA[1];
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Abstract: Alzheimer's Disease (AD) is the most common neurodegenerative disease in humans and is predicted to have an increasing societal impact in coming years. There are currently no pharmaceutical treatments proven to slow the progression of this disease. This emphasizes the need to explore novel drug targets in AD. Our lab identified Agomelatine (AGO) through a high-throughput drug screening algorithm that identifies possible treatments for AD by analyzing off-target effects of pre-existing pharmaceuticals. AGO was selected for its beneficial effect on mitigating neurodegeneration and inflammation both of which contribute to AD progression. AGO, also under the brand name Valdoxan, has been previously used to treat clinical depression.

Its mechanism of action lies in its ability to act as both an agonist for the MT1/MT2 melatonin receptors and an antagonist for the $5HT_{2C}$ serotonin receptor. This synergistic action leads to reduced neurodegeneration, inflammation, and anxiety. Our lab uses a transgenic rat model (TgF344-AD) that develops cognitive deficits and AD pathology, including extracellular amyloid plaques, tauopathy, gliosis, and neuronal loss due to the expression of the mutant human amyloid precursor protein (APPsw) and presenilin 1 (PS1ΔE9) genes. Male and female TgF344-AD and their wild type littermates were included in our studies. Oral treatment with AGO (dose 8-10 mg/kg/day), started at 5 months of age (pre-pathology) and continued for 6 months until the endpoint at 11 months of age. The effect of AGO on cognitive decline was assessed using the hippocampal dependent active place avoidance test performed at 9 and 11 months of age. Transgenic treated rats showed an improvement in learning when comparing 9 month performance to 11 month performance. This suggests AGO treatment is mitigating the cognitive decline we would expect to observe with aging of the animals. Immunohistochemistry and western blot analyses on brain tissue will determine the effect of AGO on amyloid plaques, activated microglia, tauopathy, and inflammation to determine how AGO treatment affects AD pathology. In these studies, we show that AGO rescues cognitive function in the TgF344-AD rat model. Based on our data, we propose that AGO, through its role as both an MT1/MT2 agonist and $5\text{-}HT_{2c}$ antagonist, is a drug that can be repurposed to treat AD.

Disclosures: G. Terry: None. **L. Xie:** None. **P. Rockwell:** None. **P. Serrano:** None. **M. Figueiredo-Pereira:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.09

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

Support: NIMH Grant MH109466
NIMH Grant MH109466s

Title: Effects of histone deacetylase inhibitors on memory and behaviors in app/ps1 mice

Authors: *B. MCCLARTY, H. DONG;
Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Introduction: It has been demonstrated that histone modifications regulate memory and behavior, and histone deacetylase **(**HDAC) inhibitors display promising beneficial effects in aging and Alzheimer’s disease (AD) models. However, currently there are no studies for cross comparison of pan and selective HDAC inhibitors to evaluate their effects on memory, affective

behaviors and AD-like pathologies in a temporal and spatial manner. **Methods:** In this study, we compared a pan (VPA) and two selective HDAC inhibitors (CI-994, targeting HDAC1, 2 and 3; and MS-275, targeting HDAC1 and 3) in APP/PS1 mice and age matched controls. We administered VPA, CI-994, and MS-275 and vehicle to 12-, and 18-month-old APP/PS1 mice and controls daily for 30 days (IP). Then, executive memory (novel object recognition), short-term working memory (Y maze) and the long-term spatial reference memory (Morris Water Maze (MWM)) tests, anxiety (light/dark box) and depression-like behaviors (tail suspension test) were assessed. **Results:** We found executive memory deficits in 12-month and 18-month-old (both $p<0.0001$) APP/PS1 mice. Chronic treatment of VPA, MS-275 and CI-994 in both 12-month and 18-month-old APP/PS1 mice significantly improved executive memory as compared to age matched controls (both $p<0.0001$). We found working memory deficit in 12-month and 18-month-old APP/PS1 as compared to age matched controls (both $p<0.001$). However, only APP/PS1 at 18-month of age improved working memory by VPA ($p<0.01$), CI-994 ($p<0.0001$), and MS-275 ($p<0.01$). Finally, we found spatial reference memory deficits in both 12-month ($p<0.05$) and 18-month-old ($p<0.001$) APP/PS1 mice. MS-275 ($p<0.001$) and CI-994 ($p<0.01$) could improve this memory at 18-months of age. For affective behaviors, we found 12-month ($p<0.001$) and 18-month-old ($p<0.05$). APP/PS1 mice showed increased anxiety-like behaviors when compared to age matched controls. VPA ($p<0.05$), MS-275 ($p<0.01$), and CI-994 ($p<0.01$) decreased anxiety-like behaviors in 12-month-old APP/PS1 mice, but only MS-275 ($p<0.0001$) and CI-994 ($p<0.001$) decreased anxiety like behaviors in 18-month-old APP/PS1 mice. We did not find depressive-like behaviors and drug effects in APP/PS1 mice at any age we tested.**Conclusions:** Our study indicates that APP/PS1 mice displayed variety of memory deficits in APP/PS1 mice at both 12 and 18 months of age. HDAC inhibitors, especially CI-994 and MS275 could rescue memory deficits in APP/PS1 mice with memory domain specifics and decrease anxiety behavior. Next, we will determine the impact of HDAC inhibitors on AD pathogenesis in APP/PS1 mice.

Disclosures: B. McClarty: None. **H. Dong:** None.

Poster

P232. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P232.10

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

Support: NIH/NIA R21AG056947
NIH/NIA T32AG00115
NIH/NIA P01AG9973
NIH/NINDS F31NS068219

NIH/NIGMS T32GM008541

Title: Loss of tonic inhibitory control of sharp wave ripples in the TgF344-AD rat

Authors: *M. H. RATNER[1], S. S. DOWNING[1], O. GUO[2], K. ODAMAH[1], V. KUMARESAN[1], W. XIA[3], D. H. FARB[1];
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Abstract: A better understanding of the changes in neural activity that contribute to the prodromal progression and onset of overt symptoms of Alzheimer's disease (AD) is essential to the discovery of effective therapeutics. These experiments explore prodromal neural network dysfunction in the hippocampal trisynaptic circuit by using an orally bioavailable established memory enhancer (α5IA) that acts as a selective negative modulator of extrasynaptic α5GABA-A receptors in TgF344-AD transgenic rats which express human amyloid-beta precursor protein (with the Swedish mutation) and human presenilin-1 (with a Δ exon 9 mutation). The results demonstrate that oral administration α5IA (1.0 mg/kg) increases CA1 pyramidal cell mean firing rates and peak CA1 ripple amplitude during wakeful immobility in wild type F344 rats. By contrast, the ripples of young adult TgF344-AD rats are nonresponsive to α5IA indicating network dysfunction prior to the onset of plague formation but when oligomers have been shown to be elevated in the brain. We also show that TgF344-AD rats, exhibit high serum Aβ42 and Aβ40 levels by 3 months of age. These results demonstrate, to the best of our knowledge, the first evidence for prodromal α5GABA-A receptor dysfunction in the AD hippocampal trisynaptic circuit of this rat model. Moreover, as α5GABA-A receptors are located extrasynaptically and subserve the function of tonic inhibition we posit that an early stage of memory dysfunction involves the disruption of tonic inhibition in the hippocampus.

Disclosures: M.H. Ratner: None. **S.S. Downing:** None. **O. Guo:** None. **K. Odamah:** None. **V. Kumaresan:** None. **W. Xia:** None. **D.H. Farb:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.01

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: American Heart Association (AHA)–Allen Initiative in Brain Health and Cognitive Impairment Project 19PABH134580006 (to S.H.S. , B.D. P. and associates)
Medical Research Council, United Kingdom (MR/S002626/1 to M.W.)

the Brian Ridge Scholarship (R.T.)
European Research Council under the European Union's Horizon 2020 research and innovation programme (864921 to M.R.F.)
US Public Health Service Grant DA044123 (to S.H.S.)

Title: Neuroprotective functions of the ancient signaling molecule, hydrogen sulfide, in Alzheimer's disease

Authors: *B. D. PAUL[1], D. GIOVINAZZO[1], B. BURSAC[2], R. TORREGROSSA[3], M. WHITEMAN[3], M. R. FILIPOVIC[2], S. SNYDER[1];
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Abstract: Alzheimer's disease (AD) is a devastating, progressive neurodegenerative disease that causes loss of memory and executive function, and is the leading cause of dementia worldwide. Neuropathologic hallmarks of AD include deposition of amyloid plaques, neurofibrillary tangles (NFTs) and paired helical filaments in the brain. Mutations in the microtubule-associated protein Tau, a component of the NFTs, cause its hyperphosphorylation and aggregation in AD. Here we show that cystathionine γ-lyase (CSE), the biosynthetic enzyme for the gaseous messenger molecule, hydrogen sulfide (H_2S), binds wild type Tau, which enhances its catalytic activity. By contrast, CSE fails to bind Tau P301L, a mutant that is present in the 3xTg-AD mouse model of AD. We further demonstrate that signaling by H_2S is dysregulated in AD. H_2S signals via a posttranslational modification termed sulfhydration/persulfidation, wherein -SH groups of reactive cysteine residues are converted to -SSH groups. Sulfhydration regulates a plethora cellular processes ranging from response to inflammation to cardiovascular function. Using the newly developed dimedone switch assay, we show that sulfhydration is diminished in 3xTg-AD mice as well as in human AD brains. Furthermore, H_2S prevents hyperphosphorylation of Tau by sulfhydrating its kinase, glycogen synthase kinase 3β (GSK3β) and inhibiting its catalytic activity. Finally, we demonstrate that sulfhydration is diminished in AD, while administering the H_2S donor sodium GYY4137 (NaGYY) to 3xTg-AD mice ameliorates motor and cognitive deficits in AD. Aging is a risk for developing AD and we further show that H_2S signaling and sulfhydration is compromised during aging, which leads to impaired stress responses.

Disclosures: B.D. Paul: None. **D. Giovinazzo:** None. **B. Bursac:** None. **R. Torregrossa:** None. **M. Whiteman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Exeter. **M.R. Filipovic:** None. **S. Snyder:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: 1 RF1 AG058778

Title: Regulation of cholesterol biosynthesis by the ER stress-sensor, PERK, and its implications for neurodegeneration

Authors: *R. VISWANATHAN[1], B. J. STOVEKEN[2], H. GUDLAVALLETI[2], S. T. WEINTRAUB[2], J. D. LECHLEITER[2];
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Abstract: Misfolded proteins are a common phenotype of many neurodegenerative diseases including Alzheimer's, frontotemporal dementia, and progressive supranuclear palsy. When misfolded proteins accumulate in the endoplasmic reticulum (ER), the unfolded protein response (UPR) is activated in an attempt to restore cellular homeostasis. Interestingly, the UPR stress-sensor, PERK, is activated in the brains of patients with neurodegenerative tauopathies. However, both positive and negative modulation of PERK activity can be therapeutically beneficial. The specific mechanisms by which PERK signaling contributes to neurodegenerative diseases need to be more clearly defined.
We performed global proteomics to identify pathways that were differentially regulated by PERK knockout (PERK-KO) in mouse embryonic fibroblasts (MEFs) using data-independent acquisition mass spectrometry (DIA-MS). Protein identification and relative quantification were accomplished with Scaffold DIA (Proteome Software). The results for comparisons among experimental groups were submitted to Reactome for pathway analysis. DIA-MS analysis of wild-type and PERK-KO MEFs identified > 5900 proteins. Cholesterol biosynthesis was the top over-represented pathway for proteins with ≥ 1.5x down relative abundance (9 of 26 entities found; entities p, 4.46E-6, entities FDR, 4.89E-3). Of note were lanosterol 14-alpha demethylase (> 4x down) and 24-dehydrocholesterol reductase (DHCR-24)(> 2x down). Cholesterol plays an important role in cellular homeostasis and is an essential component in various metabolic pathways. Its dysregulation has been linked to a variety of diseases, including cardiovascular disease, diabetes, neurodegenerative disorders and cancer. An elevation of the level of cholesterol has been shown to correlate with ER stress and activation of the stress response pathway. However, the relationship between PERK and cholesterol biosynthesis has been under-investigated. Our preliminary data suggest PERK regulates key enzymes in the cholesterol biosynthesis pathway. Experiments are underway to examine the mechanism by which PERK is influencing the cholesterol biosynthesis pathway and how this impacts pathology in a neurodegenerative disease model. Additional cell types to be studied include neurons and astrocytes. This study uncovers an important role for PERK in regulating cholesterol biosynthesis, potentially revealing a mechanistic link to various neurodegenerative diseases.

Disclosures: R. Viswanathan: None. **B.J. Stoveken:** None. **H. Gudlavalleti:** None. **S.T. Weintraub:** None. **J.D. Lechleiter:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Veterans Affairs Career Development Award Level-2 #BX004341-01A1
Veteran Affairs Merit Review Grant #I01BX002619
NIH Grant RF1AG055474

Title: Loss of aly genes (ALYREF) suppresses toxicity in transgenic Caenorhabditis elegans models of tau or TDP-43

Authors: *R. KOW[1,2], A. H. BLACK[3], A. D. SAXTON[3], N. F. LIACHKO[1,2], B. C. KRAEMER[1,2,2];
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Abstract: Both the microtubule-associated protein tau and the DNA and RNA binding protein TDP-43 accumulate into toxic aggregates in multiple neurodegenerative diseases such as Alzheimer's disease and frontotemporal dementias. We have used transgenic *Caenorhabditis elegans* models of human tau and TDP-43 toxicity to discover novel genes that modulate their toxicity. These transgenic models have significant locomotor deficits, progressive loss of neurons, shortened lifespan, and accumulation of hyperphosphorylated and insoluble tau or TDP-43 protein. We previously found that the RNA binding protein *sut-2/MSUT2/ZC3H14* is a strong suppressor of tau toxicity in *C. elegans* models as well as mammalian models. To better understand the mechanism in which *sut-2/MSUT2* suppresses tauopathy, we investigated the *aly* genes (3 genes in *C. elegans*), whose mammalian homolog ALYREF has been shown to interact with *MSUT2/ZC3H14* (Morris & Corbett (2018) Nucleic Acids Res 46:6561-6575). Additionally, research in *Drosophila melanogaster* of TDP-43 toxicity showed that loss of the fly homolog *ref1* could suppress TDP-43-induced toxicity (Berson et al (2019) Acta Neuropath Commun 7:65). Therefore we determined whether loss of *aly* genes suppressed tau and/or TDP-43 toxicity in our *C. elegans* models. We found that loss of *aly-1* or *aly-2* alone, but not *aly-3*, caused mild suppression of tau-induced locomotor deficits, while loss of *aly-1* and *aly-3* or *aly-2* and *aly-3* together caused the strongest suppression of locomotor deficits. Loss of *aly*-2 and *aly*-3 reduced total and insoluble tau protein accumulation as well as neuron loss with age in tau transgenic *C. elegans*. This was not due to a reduction in tau mRNA levels; in contrast, tau mRNA levels appeared to be increased with loss of *aly-2* and *aly-3.* In TDP-43 transgenic *C. elegans*, loss of *aly-2* and *aly-3* also suppressed behavioral deficits induced by TDP-43 but did not significantly alter total TDP-43 or phosphorylated TDP-43 protein levels. The results here

suggest that although the *aly* genes modulate both tau and TDP-43-induced toxicity, they do so via different mechanisms.

Disclosures: R. Kow: None. **A.H. Black:** None. **A.D. Saxton:** None. **N.F. Liachko:** None. **B.C. Kraemer:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.04

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: SNSF Postdoc.Mobility Fellowship to S.M.K.G. (P400PB_186800)
Rainwater Charitable Foundation Grant to K.S.K.
NINDS Grant to K.S.K. (U54 NS100717-01)
Larry Hillblom Foundation Grant to K.S.K.
NIH K99 grant to J.N.R (NIH K99: AG064116)
NINDS Grant to S.T. (R35 NS097277)
NIA Grant to S.T. (AG056293)

Title: MAPT mutations induce dysregulation of the cholesterol biosynthesis pathway in human forebrain organoids

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Abstract: Mutations in the MAPT gene, encoding tau, are well-known genetic causes of neurodegenerative diseases including Frontotemporal Dementia. However, the molecular and cellular mechanisms of MAPT mutations leading to disease are poorly understood. Brain organoids generated from patient-derived pluripotent stem cells carrying MAPT mutations offer a novel tool to track the earliest effects of MAPT mutations in a complex cellular context, including cortical pyramidal neurons and astrocytes among other cell types found in the human brain. Forebrain organoids from induced pluripotent stem cells of five different heterozygous carriers of MAPT mutations (R406W mutation: 2 individuals, V337M mutation: 3 individuals) were generated and assayed using droplet-based single-cell RNA sequencing at various timepoints during their maturation (2, 3, 4 and 6 months of age). CRISPR-corrected isogenic lines served as controls. In organoids that carry the V337M and R406W mutations, the

cholesterol biosynthetic pathway in astrocytes was the top upregulated gene set compared to isogenic controls. The 15 upregulated genes involved in cholesterol metabolism included HMGCS1, encoding the rate-limiting step for cholesterol biosynthesis, ACAT2, converting cholesterol to its storage form cholesteryl esters, and SREBF2, a master transcriptional regulator of cholesterol biosynthesis genes. Organoids generated from a homozygous R406W mutant cell line similarly showed upregulation of the cholesterol biosynthesis pathway. Furthermore, this result was confirmed by immunohistochemistry of the organoids, and by loading tau protein into primary astrocytes. Our study is the first to link MAPT mutations to altered cholesterol metabolism. Cholesterol homeostasis in the brain is tightly regulated by efflux and levels of cholesterol biosynthetic enzymes. Furthermore, cholesteryl esters can increase tau phosphorylation. Our findings thus suggest that cholesterol dyshomeostasis is an early event in the etiology of neurodegeneration caused by tau mutations.

Disclosures: S.M.K. Glasauer: None. **S.K. Goderie:** None. **J.N. Rauch:** None. **E. Guzman:** None. **M. Audouard:** None. **T. Bertucci:** None. **S. Joy:** None. **E. Rommelfanger:** None. **G. Luna:** None. **S. Lotz:** None. **S. Borden:** None. **K. Onanuga:** None. **S. Temple:** None. **K.S. Kosik:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.05

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: RF1 AG062171-01

Title: Nucleocytoplasmic Transport and Nuclear Pore Disruption in Tauopathies

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Abstract: As human life expectancy continues to increase, the two most common dementias, Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD), are becoming more prevalent with no cure and no effective medicine or significantly alter disease progression. When compared to to intercellular amyloid plaques, intraneuronal accumulations of neurofibrillary tangles constituted by insoluble, filamentous, hyperphosphorylated Tau is better correlated with neuronal loss and the clinical progression of AD. In addition, the burden of Tau aggregation is also positively associated with the progression of Frontotemporal lobar degeneration (FTLD), the pathologic diagnosis for clinical FTD. However, it remains unclear how Tau accumulation contributes to the devastating neuronal dysfunction and/or death characteristic of these disorders.

Therefore, understanding the cellular mechanisms underlying Tau-mediated neuronal toxicity during aging may provide much needed therapeutic targets. Our previous studies discovered that hyperphosphorylated Tau interacts with nucleoporins and disrupts the nuclear pore complex (NPC) (Neuron, 2018). The pathological accumulation of Tau in mice and human patients impedes nuclear transport and may disrupt the NPC. Furthermore, our studies in Amyotrophic Lateral Sclerosis (ALS) have suggested that rescuing the NPC injuries prevents various defects in human disease models. Here, we sought to determine the cellular and molecular mechanisms underlying Tau mediated nuclear pore injuries in AD and FTD-Tau. We found that nucleoporin 210 (NUP210) mislocalized to cytosol in both AD patients and the PS19 mouse model. In addition, we provide evidence suggesting that FG-repeat containing nucleoporin NUP62 directly interacts with pTau both *in vivo* and *in vitro*, whereas other nucleoporins such as NUP50, NUP54 and POM121 were not affected. Currently, we are evaluating if reducing levels of NUP210 or NUP62 in rodent disease models and induced pluripotent cell lines would reduce/delay pTau accumulation. Moreover, we will determine how loss of NUP210 and NUP62 from NPC in tauopathies specifically affect nuclear transport of protein and RNA. Eventually, we will develop mechanism-based therapy to rescue NPC defects, in order to ameliorate neuronal injury in tauopathies.

Disclosures: J. Chew: None. **L. Ruan:** None. **C. Cook:** None. **L. Petrucelli:** None. **J.D. Rothstein:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01AG042178
R01AG47812
R01NS105473

Title: A small molecule Hyperphosphorylated tau inhibitor probing for Possible Therapeutic development in Alzheimer Disease and Tauopathies

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Abstract: A small molecule Hyperphosphorylated tau inhibitor probing for Possible Therapeutic development in Alzheimer Disease and Tauopathies Jangampalli Adi Pradeepkiran[1], Arubala P Reddy[2], P. Hemachandra Reddy[1,3,4,5,61]Department of Internal Medicine,[2]Nutritional Sciences

Department,[3]Department of Pharmacology and Neuroscience, [4]Department of Neurology, [5]Department of Public Health, Graduate School of Biomedical Sciences, [6]Department of Speech, Language, and Hearing Sciences, TTUHSC, Lubbock, TX 79430, USA**Abstract**The purpose of our study is to understand the protective effects of small molecule ligands for phosphorylated tau (p-tau) in Alzheimer's disease progression. Many reports evidenced that phosphorylated tau (p-tau) is reported to be an important contributor to the paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) formation in Alzheimer Disease (AD).**Methods:** In the present study, we rationally selected and validated small molecules (ligands) for p-tau that binds to the phosphorylated tau at SER23 (Ser 285) responsible for GSK3β, CDK5 and TYR44 (Y310) DYRK1A, which cause phosphorylated kinase upregulation in AD. In our present study, we assessed the molecular dynamics, and validated molecular dockings for the three best ligands. Based on docking scores -8.09, -7.9 and -7.8kcal/mol, we found that ligand 1 binds to the key hyperphosphorylation residues of p-tau and predicts to inhibit abnormal PHF-tau, DYRK1A, and GKS3β that reduce p-tau levels in AD. Using biochemical, molecular, immunoblotting and immunofluorescence transmission electron microscopy, analyses, we studied the p-tau ligand inhibition and mitochondrial protective effects against mutant tau induced synaptic and mitochondrial toxicities in hippocampal neuronal (HT22) cells. **Results:** We found interaction between NAT10-262501 (ligand 1) and p-tau at key phosphorylation sites and these ligand-based inhibition decreased PHF-tau, DYRK1A, and GSK3β levels. We also found increased mitochondrial biogenesis, mitochondrial fusion and synaptic activity and reduced mitochondrial fission in ligand 1-treated mutant tau HT22 cells.**Conclusions:** Based on these results, we cautiously conclude that p-tau NAT10-262501 (ligand 1) reduces hyperphosphorylation of p-tau based CDK5 and GKS3β kinase regulation in AD, and helps to maintains neuronal structure, mitochondrial dynamics and biogenesis with a therapeutic function in AD.***Key words*:** Hyperphosphorylated tau, small molecules, PHF-tau, Alzheimer Disease.

Disclosures: P. Jangampalli Adi: None. **A.P. Reddy:** None. **P.H. Reddy:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01AG066211

Title: Exploring mechanisms for xbp-1s-mediated suppression of tauopathy in C. elegans

Authors: S. M. WALDHERR[1], ***M. HAN**[2], A. SAXTON[1], M. BAUM[1], T. STROVAS[1], P. MCMILLAN[1], J. WHEELER[1], B. KRAEMER[1];

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Abstract: Protein homeostasis (proteostasis) is required for neuronal function over the lifespan. Disrupted proteostatic mechanisms, such as the unfolded protein response (UPR), are associated with protein aggregation and neurodegeneration. The UPR contains three branches: PERK, IRE1, and ATF6. Collectively, they regulate translational capacity and ER-associated degradation in response to ER stress. Previous work has shown that XBP-1s, a transcription factor downstream of IRE1, mediates suppression of disease phenotype in a *C. elegans* model of human tauopathy through a mechanism requiring ATF6. To identify the molecular mechanisms of this phenotypic rescue, we conducted a transcriptomic analysis of *xbp-1s* gain-of-function versus non-transgenic *C. elegans*. We found five upregulated human gene homologs that are also associated with ATF6: *csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lipl-3*. CSP-1, DNJ-28, HSP-4, and LIPL-3 loss of function abolished *xbp-1s*-mediated rescue of tauopathy phenotype, proving they are necessary for *xbp-1s*-mediated tauopathy suppression. Further, *xbp-1s*-mediated reduction of tau accumulation requires functional *hsp-4*, a *C. elegans* homolog of BiP. Also, *hsp-4/BiP* loss of function exacerbates behavioral phenotype of a low-expressing tauopathy model, while *dnj-28* loss of function does not. Interestingly, low level neuronal expression of *hsp-4/BiP* can mildly suppress tauopathy behavioral phenotype without *xbp-1s* overexpression, but high level neuronal overexpression of *hsp-4/BiP* is not well tolerated. While investigation of other *xbp-1s* target genes is still in progress, we have thus far presented a novel mechanism of *xbp-1s*-mediated suppression of tauopathy involving *hsp-4/BiP*. Understanding the molecular mechanisms responsible for UPR-mediated changes in tauopathy can inform mammalian studies and eventually lead to more precisely targeted treatments for neurodegenerative disease.

Disclosures: S.M. Waldherr: None. **M. Han:** None. **A. Saxton:** None. **M. Baum:** None. **T. Strovas:** None. **P. McMillan:** None. **J. Wheeler:** None. **B. Kraemer:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01-AG60766

Title: Role of alpha-synuclein and caspase-2 mediated cleavage of tau in LTD

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Abstract: The neurons of patients with Lewy body Diseases (LBD) often contain high levels of misfolded tau protein and α-synuclein, and many patients develop dementia. Caspase-2 is a cysteine-aspartic protease that has been shown to mediate long-term depression (LTD), a form of synaptic plasticity characterized by the internalization of AMPA receptors, which leads to synaptic weakening that is believed to underlie forgetting. Recently, we found that in mice, caspase-2 cleaves tau at aspartate 303, and this process mediates LTD (unpublished data). The A53T α-synuclein variant linked to familial Parkinson's disease increases the internalization of AMPA receptors in a tau-dependent manner, but the role of caspase-2 and the effect of this process on LTD are unknown. Here, we provide evidence to test the hypothesis that in LBD caspase-2 cleavage of tau leads to excessive synaptic weakening. We measured LTD in hippocampal slices from transgenic mice expressing A53T α-synuclein and non-transgenic littermates in which mouse tau was wild-type or mutated (aspartate-303-glutamate) to render tau resistant to caspase-2 cleavage. Our preliminary data indicate that in mice with wild-type tau, A53T α-synuclein is associated with enhanced LTD, supporting our hypothesis that there is excessive synaptic weakening in LBD. We are obtaining LTD measurements in mice with caspase-2 resistant tau to determine if this enhanced LTD depends on caspase-2 cleavage of tau.

Disclosures: Z. Crabtree: None. **K. Ashe:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.09

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant AG605925

Title: Activity- and Phosphorylation-dependent Release of Endogenous Tau from Human Neural Progenitor Cell line ReNCell VM

Authors: G. SINDI[1], R. A. COLVIN[2], ***D. LEE**[1];
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Abstract: A key pathological hallmark of Alzheimer's disease (AD) and related dementia is the formation of neurofibrillary tangles (NTFs), which are aggregates of hyper-phosphorylated tau. Tau is a microtubule-associated protein but, if hyper-phosphorylated, it becomes pathogenic causing tauopathies including AD. Tau is an intracellular protein but also known to be released to the extracellular fluid. Released tau can have both physiological and pathological roles in the nervous system, but its release mechanism remains to be further explored. Tau undergoes several posttranslational modifications and phosphorylation is the most commonly observed. Phosphorylation of specific tau phosphorylation sites (pSites) is important for its association with

microtubules and normal function, but its hyper-phosphorylation is a common feature of tauopathies. Tau phosphorylation is also known to increase its release from neurons. Several studies have shown that tau release can be activity-dependent. Interestingly, disruption of neuronal activity is involved in AD pathogenesis. Therefore, the impact of activity- and phosphorylation-dependent release of tau on tauopathy is of significant interest as disruption of neuronal and synaptic activity is involved in AD pathogenesis. In this study, we developed an in vitro model to study activity-dependent tau release using immortalized human *ReNCell VM* line, which was derived from the fetal mid-brain and recently used to study pathological mechanisms underlying AD. *ReNCell VM* line was differentiated into neurons for 14 days in vitro to study activity-dependent tau release. Our results showed that differentiated human neurons express tau, which can be released by KCl-induced depolarization (50mM for 1 hr). We are currently studying how tau phosphorylation contributes to its activity-dependent release. Since a protein kinase GSK-3β is known to phosphorylate multiple tau pSites, we used a selective GSK-3β inhibitor SB216763 (10μM, 2 hrs). Our results showed that SB216763 decreased activity-dependent tau release compared to untreated neurons. Lastly, a selected group of pSite-specific tau antibodies (e.g., AT8) has been used to examine the phosphorylation profiles of extracellular tau released by neuronal stimulation. We found that released tau is phosphorylated at multiple pSites in proline-rich domain and C-terminal. Taken together, *ReNCell VM* showed activity- and phosphorylation-dependent release of endogenous tau and thus can be used to study the mechanism underlying activity-dependent tau release and its propagation in AD.

Disclosures: G. Sindi: None. **R.A. Colvin:** None. **D. Lee:** None.

Poster

P284. Cellular and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P284.10

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R01 Grant 1R01NS119280

Title: The role of tau interactor in the propagation of tau-seed and neurodegeneration.

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Abstract: Tau aggregates are critical pathological features of Alzheimer's disease (AD) and other tauopathies. An important focus of research has been to understand the pathological tau propagation in AD patient brains that follow neuronal networks. Despite the knowledge acquired, the cellular mechanism involved in tau propagation and seeding are still unclear. Unfortunately, the nature of the tau species involved in the spreading and the precise seeding/template remains unknown. Despite this uncertainty, some studies suggest that a high molecular weight tau (HMW-tau) is the form of tau involved in trans-synaptic propagation. It remains unresolved whether these HMW-tau particles are made exclusively of tau or contain other constituents such as proteins necessary for propagation. Here, by using a 3-months old PS19 mouse model harboring the human tau^{P301S}, we developed a strategy to identify proteins that interact with tau in PS19 mice, as well as in human AD and PSP cases. TBS-soluble brain extracts were passed through a Size Exclusion Chromatography (SEC) column. We then measured the tau seeding activity of each SEC-fraction using a well-described bio sensor cell line that relies on flow cytometry detection of FRET signal. We determined that tau from fraction-9 (F9) forms an HMW-tau complex *in vivo* (>2,000KDa) with the strongest seeding activity. Interestingly, the tau present in F9 is less than 4.4% of the total tau in the brain of PS19 mice and adopt a protofibrillar morphology. To identify other constituents or interactors of this tau- seed, we IP human tau using the HT7 antibody from fraction-9 and subjected it to TMT-tag quantitative Mass Spectrometry. Through quantitative Mass Spectrometry, we were able to identify the protein-interactome of the tau- seed and the interactome of monomeric tau isolated from the same brains. Bioinformatics from both interactomes revealed an enrichment of a specific set of proteins in the tau-seed in comparison with monomeric tau. To further analyze the HMW tau-seed interactome, we developed a strategy using SEC and the biosensor cell line to detect seeding activity in a *Drosophila* model of tauopathy, that will allow us to screen critical interactors that confer the tau seeding ability and spreading.

Disclosures: P. Martinez: None. **H. Patel:** None. **Y. You:** None. **N. Jury:** None. **A. Perkins:** None. **Y. You:** None. **X. Huang:** None. **S. Dutta:** None. **A. Wijeratne:** None. **J. Redding:** None. **A. Mosley:** None. **J. Rochet:** None. **J. Zhang:** None. **J.C. Troncoso:** None. **C. Lasagna-Reeves:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21HDO96309

Title: Early Developmental Response to Prenatal of a Novel Rat-Adapted Zika Virus

Authors: E. LEMANSKI[1], ***J. MELCHIORRE**[1], M. PARCELLS[2], J. M. SCHWARZ[3];
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Abstract:

Disclosures: E. Lemanski: None. **J. Melchiorre:** None. **M. Parcells:** None. **J.M. Schwarz:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5P20GM103642

Title: The Cholinergic Anti-Inflammatory Response in High Fat Diet induced Neuroinflammation

Authors: S. COTTO-RÍOS[1], C. LÓPEZ[4], J. LASALDE-DOMINNICCI[2,3,6,7], ***J. COLÓN-SÁEZ**[5];
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Abstract: Obesity rates have nearly tripled in the past 45 years affecting globally close to 2 billion adults which are either overweight or obese1, cocurrently, the worldwide prevalence of dementia has also tripled in that same span2. One of the mechanisms that is thought to play a major role in obesity induced cognitive decline is the consumption of a High Fat Diet (HFD). HFD has been shown to activate neuroinflammation, which can result in neuronal injury3, and can lead to a decline in cognitive performance5,9-11. Neuroinflammation has been shown to be a common denominator of many neurodegenerative and neuropsychiatric disorders8. Diet induced neuroinflammation, is mediated by activation of the CNS resident immune cells, the microglia3-7. Recently an important role for the alfa-7-nicotinic acetylcholine receptor (α7-nAChR) has been found in the control of both systemic13,14 and CNS inflammation15-17. This receptor is a

key component of the cholinergic anti-inflammatory response which is a neural system that controls both systemic and neuroinflammation. To study the therapeutic potential of the α7-nAChR for diet induced neuroinflammation we used the allosteric modulator PNU-120596. Mice were fed either a control or a HFD and their cognitive performance was characterized using the novel location recognition test. Furthermore, the activation of neuroinflammation was also studied using western blot and ELISA. Our data indicate that mice that received PNU-120596 had no significant difference to control groups that received either the control or the HFD alone. However, both western blot and ELISA analysis shows that PNU was able to prevent the inflammatory changes that result from the HFD. So far, we can conclude that PNU is effective at reducing the inflammatory response to an HFD, however we could not observe cognitive effects of this diet under our experimental conditions.

Disclosures: S. Cotto-Ríos: None. **C. López:** None. **J. Lasalde-Dominnicci:** None. **J. Colón-Sáez:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1RF1AG059610-01
NIH Grant 1RF1AG057882-01
NIH Grant 5R01NS118747-02
NIH Grant 5R01MH106490-05

Title: Minimal detection of microglia-derived extracellular vesicles in different in vivo pathological models in the CNS

Authors: *X. CHEN[1], S. JIN[3], J. DUNNELL[4], Y. YANG[2];
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Abstract: Microglia are resident immune cells responsible for surveying the parenchyma and clearing debris/invaded pathogens in the CNS. Activated microglia contribute to elevated neuroinflammation and subsequent disease pathology. In recent years, microglia-derived extracellular vesicles (EV) and especially exosomes (size 50-200 nm) that are either shed from plasma membrane or originated from multi-vesicular body (MVB), have been implicated to propagate disease-causing protein formed aggregates, potentially contributing to disease progression. Currently, most if not all studies employ culture models and ultracentrifugation technique to isolate microglia-derived EVs which have been shown to be often associated with

secreted proteins. In the present study, by employing size-exclusion chromatography (SEC), we first purified microglia-derived exosomes from primary cultured wild type (WT) and SOD1G93A microglia. Immunoblotting of exosome markers shows that SEC-based purification is able to clearly separate exosomes from microglia-secreted proteins. To examine how immunoactivated microglia may alter their EVs secretion and property, both WT and SOD1G93A^{+} microglia were treated with LPS, which found selectively increased release of CD63^{+} exosomes from WT but not SOD1G93A^{+} microglia based on fluorescence Nanoparticle tracking analysis (fNTA). Although microglia-derived EVs have been implicated in propagating protein aggregates, it has not been directly characterized in vivo. To further characterize microglia-derived EVs in vivo, we bred our recently generated cell-type specific exosome reporter CD63-GFP$^{f/f}$ mice with Cx3cr1-CreER^{+} mice to selectively label microglia-derived EVs/exosomes following 4-OHT injections. Surprisingly, we observed vast majority (>95%) of induced CD63-GFP puncta signals inside Iba^{+} microglia with only sparse extracellularly localized CD63-GFP signals. Subsequent LPS injections (i.p.) have no effect in stimulating extracellular localization of microglia-derived CD63-GFP^{+} puncta signals even microglia have become highly activated. We further generated Cx3cr1-CreER^{+}CD63-GFP$^{f/+}$SOD1G93A^{+} mice to examine the extracellular localization of microglia-derived EVs in ALS model. Consistently, we observed minimally extracellularly localized microglia-derived EVs. Taken together, although cultured microglia secrete typical EVs/exosomes, microglia-derived EVs are only minimally observed in the exosome-reporter model in different physiological and pathological conditions. Our study calls for the need for further characterization of microglia derived EVs in vivo in the CNS.

Disclosures: X. Chen: None. **S. Jin:** None. **J. Dunnell:** None. **Y. Yang:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Spanish Ministerio de Economía y Competitividad Grant #SAF2017-84283-R
CIBERNED Grant #CB06/05/2004 .

Title: Neuroprotective role of Licochalcone-A in lipopolysaccharide-induced neuroinflammation mice model

Authors: M. CARRASCO[1,2,3,4], T. ESPINOSA-JIMENEZ[1,2,3], *M. E. UREÑA-GUERRERO[5], J. CASTAÑEDA-CABRAL[5], C. BEAS-ZARATE[5], C. AULADELL[2,3,6], E. VERDAGUER[2,3,6], J. FOLCH[2,4], A. CAMINS[1,2,3], M. ETTCHETO[1,2,3];
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Abstract: Neuroinflammation has shown to play a key role in the development of neurodegenerative disorders, specifically in cognitive decline. For this reason, the inhibition of this process could represent a potential therapeutic target for these diseases. Recently, Licochalcone-A (LCA), a chalcone extracted from the root of *Glycyrrhiza glabra,* has shown multiple therapeutical properties including anti-inflammatory effect. This highlights its potential preventive effect reducing the inflammation responsible of cognitive dysfunction. However, the biological mechanism underlying the relationship between LCA and cognitive decline is still unknown. At the same time that no effective treatment able to stop the neurodegeneration has been found to date. Therefore, the aim of the present study is focused on analysing the role of inflammation in cognition and the potential preventive effect of LCA in C57BL6 mice treated with Lipopolysaccharide (LPS), a neuroinflammation mice model. To perform this study, six-week-old male mice were intraperitoneally treated with LCA at a dose of 15 mg/kg/day 3 times per week for two weeks. The last day of treatment the subjects received a final injection of 1 mg/kg of LPS, 24 hours before sacrifice. Therefore, mice were divided into 4 different experimental groups, classified in mice treated with saline or LCA and receiving or not the last LPS injection. Before sacrifice, short term memory and depression were evaluated by Novel Object Recognition test and Forced Swimming test, respectively. Afterwards, the expression of mRNA and protein levels of molecules related to inflammation, cognitive decline and dendritic spine analysis among others, were analysed in hippocampus. Our results corroborated that LPS administration induced neuroinflammation, which was effectively prevented by LCA treatment. Moreover, mice pre-treated with LCA also showed a significant improvement of memory decline and depression state in comparison to those only exposed to LPS. In agreement, LPS administration demonstrated a significant decrease in the number of dendritic spines which was ameliorated with LCA pre-treatment, in accordance with the results obtained in other analysed biomarkers. In conclusion, our results demonstrated that preventive treatment with LCA confers neuroprotection against pathological mechanisms induced by LPS, placing LCA as a potential preventive treatment for neuropathological diseases where neuroinflammation plays a key role.

Disclosures: M. Carrasco: None. **T. Espinosa-Jimenez:** None. **M.E. Ureña-Guerrero:** None. **J. Castañeda-Cabral:** None. **C. Beas-Zarate:** None. **C. Auladell:** None. **E. Verdaguer:** None. **J. Folch:** None. **A. Camins:** None. **M. Ettcheto:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Defense Advanced Research Projects Agency
NIH

Title: Social buffering reduceslpsinduced neuroinflammatory distress associated behaviors in mice

Authors: *S. SHOKAT FADAEI[1], H. WEI[3], M. BRAVO[4], A. ADEWAKUN[2], J. TALBOT[1], D. R. LITTMAN[5], Y. Z. WADGHIRI[6], R. C. FROEMKE[7,8];
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Abstract: The increase of lipopolysaccharide (LPS) concentration in the brain leads to neuroinflammation, a major determinant for cognitive impairment in many neurodegenerative diseases (Ransohoff, 2016; Zhao, 2019). Levels of LPS in the gut flora can be correlated with LPS levels in the central nervous system in patients with schizophrenia, Parkinson's disease, and Alzheimer's disease (Bhattacharyya, 2020). Here we studied the effects of systemic LPS injection (e.g., to mimic intestinal infection) on behavior.We first asked if and how much LPS would enter the central nervous systems after systemic injection. We used LPS conjugated to a far-red emitting dye (Cy5.5) and imaged LPS trafficking from the gut to the nervous system in live C57Bl6, albino, and nude adult mice. To optimize imaging from the abdominal region, mice were fed a diet without chlorophyll to decrease autofluorescence. After clearing autofluorescence, mice were injected with an LPS-Cy5.5 (N=5) or saline (N=5). In the periphery, we found that the LPS localized in the spleen, kidney, and colon. We also found that conjugated LPS could pass the blood-brain barrier and accumulate in the hippocampus.We then asked how LPS injection affected the overall behavioral profiles of isolated and socially-housed mice, using a system we developed for continuous 24/7 behavioral monitoring and neural recording (Carcea et al., 2021). Mice demonstrated a range of sickness behaviors (Hart, 1988), including reductions in arousal level, less time spent foraging, and significant time spent hunched over. Continuous recordings of videos for 24 hours pre-injection and over 72 hours post-injection were scored for LPS-injected socially-housed mice in which one of the two animals was injected (N=5), compared to saline-injected socially-housed controls (N=3), LPS-injected singly-housed mice (N=6), and saline-injected singly-housed mice (N=3). We found that co-housing an infected mouse with a healthy conspecific reduced mortality and shortened the duration of expressed sickness behaviors. Thus LPS might enter the brain to directly impact neural processing and behavior, interacting with internal state factors that might be positively regulated by social buffering or social interactions.

Disclosures: S. Shokat Fadaei: None. **H. Wei:** None. **M. Bravo:** None. **A. Adewakun:** None. **J. Talbot:** None. **D.R. Littman:** None. **Y.Z. Wadghiri:** None. **R.C. Froemke:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21HD096309

Title: A Magnetic Resonance Imaging Study of Prenatal Zika Virus Infection Using a Novel Rat Adapted Zika Virus

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Abstract: Zika Virus (ZIKV) is a flavivirus that, in cases of in utero fetal infection, is known to cause microcephaly and other neurological and developmental disorders. Like humans, rats exhibit immunosuppression during pregnancy which allows the Zika Virus to infect fetuses in utero. Building on our previously developed model of prenatal ZIKV infection, we are currently using a novel rat-adapted virus that has been passaged on rat cochlear microglial cells (Mocha). This virus is expected to produce a stronger infection in rats than a human-derived virus and will possibly be more neuro invasive. Pregnant rats were infected at gestational day 18 as previously established by our model. They were inoculated either with the virus, a diluent control, a UV-inactivated virus (iZIKV), or a non-neutralizing antibody combined with the virus. This antibody was included to increase infection in vivo through antibody-dependent enhancement (ADE). This process can drive entry of the virus into cells that express an Fc receptor. Fc receptors are expressed on immune cells and are involved in antibody recognition. Nonpregnant female rats were also inoculated with diluent, iZIKV, ZIKV, or antibody+ZIKV. Febrile response was measured for seven days post-injection. This was to replicate prior results in which non pregnant rats exhibited an increase in temperature following ZIKV inoculation that pregnant females did not. This is likely due to the natural immunosuppression in pregnancy. These results will also allow us to identify the timeline of infection in the adult animals. Pups were born to pregnant females at five days post-injection. At postnatal day 2 (P2), one male and one female were selected from each litter for ex-vivo structural magnetic resonance imaging (MRI). Regions associated with cognitive and motor deficits common in prenatal ZIKV infection were chosen for assessment. Cortex, hippocampus, and cerebellum will be segmented and volume relative to overall brain size will be compared. Animals exposed to either ZIKV or antibody+ZIKV are expected to have overall decreased brain volume as well as possible decreased relative volume of these regions. These findings will validate our model as etiologically relevant for ZIKV induced microcephaly and will allow us to identify which brain areas are more affected by the virus. In

the future, we plan on using immunohistochemistry to validate any observed volumetric differences as well as quantify amount of apoptosis in the cortex and hippocampus.

Disclosures: E. Lemanski: None. **J. Melchoirre:** None. **M. Parcells:** None. **J.M. Schwarz:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS107853

Title: Macrophage-specific expression of 18 kDa translocator protein (TSPO) regulates acute neurological outcomes in a mouse model of intracerebral hemorrhage

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Abstract: Spontaneous intracerebral hemorrhage (ICH) is a stroke subtype with devastatingly high morbidity and mortality rates. ICH has complex pathophysiology and as there are no effective treatments for ICH, it is imperative to identify molecular targets for therapeutic intervention. Employing an in-vitro approach, previous studies from our laboratory demonstrated that TSPO could regulate inflammatory brain response after ICH. Moreover, ICH resulted in profound induction of TSPO in brain-infiltrating macrophages and microglia, the cells that contribute to neuroinflammation. However, despite the prevalence of TSPO in neuroinflammatory response, the precise functional role of TSPO remains largely enigmatic. To investigate the role of TSPO in macrophages, we developed a novel animal model, macrophage-specific conditional knock out (TSPOfl/fl: LysMCre), using Cre-Lox technology. TSPOfl/fl: LysMCre mice exhibited augmented neurological deficits and neurodegeneration as opposed to TSPOfl/fl mice, the experimental control, at 3 days post-ICH. Furthermore, TSPOfl/fl: LysMCre mice demonstrated enhanced hematoma volume measured using susceptibility-weighted magnetic resonance imaging and hemoglobin assay compared to control. Also, TSPOfl/fl: LysMCre mice exhibited increased brain expression of proinflammatory cytokine IL-1β in comparison to TSPOfl/fl mice at 3 days post-ICH. Altogether, the data implicate that macrophage-specific expression of TSPO after ICH could be an intrinsic mechanism to regulate exacerbated brain damage, warranting further investigation.

Disclosures: S. Sukumari Ramesh: None. **F. Bonsack:** None. **R. Dasari:** None. **A. Thomas:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: P50 AT008661-01
U19 AT010835
Altschul Foundation

Title: Neuronal stress induces inflammasome mediated microglial reactivity in a model of c9orf72 FTD/ALS

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Abstract: Background: Frontotemporal dementia and amyotrophic lateral sclerosis (FTD/ALS) are most commonly caused by G_4C_2 repeat expansions in the promoter region of the *c9orf72* gene. Produced from these expansions, dipeptide repeat proteins (DPR) have multiple negative effects on cellular homeostasis, producing neuroinflammation and cellular death. Arginine containing aggregates are amongst the most toxic, and Glycine-Arginine (GR) are significantly present in brain regions associated with FTD/ALS. Here, we identify a mechanism by which neuronal stress induced by the C9orf72 DPR, GR, can induce an inflammasome mediated reaction in microglia. Targeting this mechanism provides significant protection from musculoskeletal impairment, cognitive function, and death *in vivo*.**Methods:** Intracerebroventricular injection with either AAV9-GFP or AAV9-GFP$(GR)_{100}$ was performed on postnatal day 0 wild-type C57BL6/J and *Nlrp3* deficient (*Nlrp3*$^{-/-}$). At 14 weeks, animals were tested for motor function and memory, and were humanely euthanized for tissue collection. Microglia density and activation were measured by immunofluorescence and 3D reconstruction of Iba1 immunopositive cells. qPCR for CXCL10, a regulator of chemotaxis and inducer of proinflammatory phenotypes of microglia, was performed on cortical tissue. Next, primary neurons were transfected with GFP-$(GR)_{100}$ and CXCL10 was measured via ELISA. Primary microglia were next incubated with concentrations of CXCL10, and the resultant production of IL-1β was measured via ELISA.**Results:** Wild-type GFP-$(GR)_{100}$ mice display significant motor impairment, contextual memory deficits, and eventually significant mortality. Microgliosis and activation of microglia was present in the cortex, along with an increase in cleaved caspase-1 and IL-1β. CXCL10 was found to be highly upregulated in the cortex in GFP-$(GR)_{100}$. Genetic ablation of *Nlrp3* provided significant protection to motor impairment, contextual memory deficits, and mitigated mortality seen in wild type GFP-$(GR)_{100}$, coinciding with protection of the

cleavage of caspase-1. *In vitro*, primary neurons transfected with GFP-$(GR)_{100}$ significantly produced CXCL10 compared to GFP controls. Primary microglia incubated with CXCL10 exhibited a significant production of IL-1β.**Conclusions:** Neurons in C9orf72 FTD/ALS can induce inflammasome activation in microglia. Targeting these responses provides significant phenotypic protection, including the protection of muscle function, cognitive function, and mortality. These data implicate this neuronal-microglial crosstalk mechanism as a novel target for the treatment of FTD/ALS.

Disclosures: K. Trageser: None. **C. Smith:** None. **U.H. Iqbal:** None. **U. Raval:** None. **G.M. Pasinetti:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Lpa_1 receptor antagonists for remyelination in multiple sclerosis

Authors: *K. LORRAIN[1], M. M. POON[2], A. R. BROADHEAD[2], K. J. STEBBINS[2], J. ROPPE[3], T. SCHRADER[3], Y. XIONG[3], A. CHEN[3], D. S. LORRAIN[4];
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Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. Current treatments focus on suppressing the immune system to limit inflammation and the further loss of the myelin sheath. The next advance in the treatment of MS has focused on molecules that regulate remyelination. Lysophosphatidic acid (LPA) is a naturally occurring inflammatory lipid and its dysregulation has been implicated in inflammatory diseases, such as MS. Recent studies have shown that MS patients have elevated levels of LPA in serum and in the cerebrospinal fluid. Furthermore, LPA was observed to significantly increase in patients during disease flares compared to periods of remission. We believe this elevation drives local inflammation and limits remyelination through activation of LPA1 receptors located on oligodendrocyte precursor cells (OPCs). In early validation studies we first showed that an LPA1 antagonist, AM152 (BMS-986020) produced robust effects in OPCs driving them towards differentiation and expression of myelin basic protein. Based on these results we initiated a medicinal chemistry effort to discover a novel brain penetrant LPA1 antagonist. These efforts led to the identification of OPC2791. Significantly, this compound produced robust effects in a diverse set of in vitro assays, including OPC differentiation, cortical myelination, and organotypic brain slice. These data suggest that LPA1 receptor antagonists show promise as inducers of oligodendrocyte differentiation and maturation.

Disclosures: K. Lorrain: None. **M.M. Poon:** None. **A.R. Broadhead:** None. **K.J. Stebbins:** None. **J. Roppe:** None. **T. Schrader:** None. **Y. Xiong:** None. **A. Chen:** None. **D.S. Lorrain:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.10

Topic: C.03. Parkinson's Disease

Support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 2018/18695-9)
Hospital Sírio Libanês

Title: Unraveling the overestimated role of striatal dopamine in the subthalamic stimulation in hemiparkinsonian rats

Authors: *A. CAMPOS[1], M. CARVALHO[2], R. C. MARTINEZ[3], E. T. FONOFF[4], C. HAMANI[5], R. L. PAGANO[6];
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Abstract: Parkinson's disease (PD) is a progressive and incurable neurodegenerative disease and the deep brain stimulation (DBS) of the subthalamic nucleus (STN) is currently the gold-standard *symptomatic* treatment for patients with levodopa therapy-induced complications. However, even though the efficiency of DBS is well recognized, to this date, it is not clear how the treatment with subthalamic stimulation modulates the debilitated dopaminergic system in PD. Hence, the aim of this study was to investigate the effect of STN-DBS on the striatal dopaminergic system in a rodent model of PD. For this, male Wistar rats were injected with 6-hydroxydopamine (6-OHDA) neurotoxin, implanted or not with plastic guidance cannula in the left striatum and stainless-steel electrodes in the left STN. Seven days after the surgery, the nigrostriatal lesion was validated by apomorphine-induced rotational behavior. Six-OHDA+DBS ON animals were stimulated 2 hours a day for 5 days (130 Hz, 60 µs, 0.1 mA). Saline, 6-OHDA and 6-OHDA+DBS OFF animals were used as control. After the last stimulation, animals were reevaluated in apomorphine-induced rotation and evaluated in the immobility test. Fresh and fixed striatum were collected 13 days after the surgical procedures. For microdialysis assay, samples of intracerebral extracellular fluid were collected before, during, and after the 1st and 5th DBS session, or 8 and 12 days after the surgical procedures in the control animals. We confirmed our PD model by decreased expression of tyrosine hydroxylase (TH) in the substantia

nigra. The nigrostriatal lesion also induced motor deficits, decreasing of TH labeling, and increasing D2R expression in the striatum. STN-DBS treatment reversed the motor deficit and reduced the dopamine concentration during and especially after the first and last stimulation. Furthermore, STN-DBS treatment inhibited the DR2 overexpression without interfering with DR1 levels. Our results suggest, so far, that STN-DBS treatment induces a modulation in the dopaminergic circuitry mediated principally by indirect striatal pathway, without induces a neuroprotective effect of the remaining dopaminergic neurons.

Disclosures: A. Campos: None. **M. Carvalho:** None. **R.C. Martinez:** None. **E.T. Fonoff:** None. **C. Hamani:** None. **R.L. Pagano:** None.

Poster

P301. Neuroinflammation: Animal Model

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P301.11

Topic: F.04. Stress and the Brain

Support: Oklahoma Health Research Program (HR 18-033)
Oklahoma State University Center for Health Sciences, Intramural funds (RLD

Title: β-funaltrexamine modulates lipopolysaccharide-induced inflammation and sickness behavior in male c57bl/6j mice

Authors: *S. MYERS, D. BUCK, K. MCCRACKEN, J. T. CURTIS, R. L. DAVIS;
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Abstract: Neurological disorders often involve inflammation in the brain, as well as in the periphery. Considering treatment options, many of the medications available do not have potent anti-inflammatory properties. Consequently, identification of novel therapeutic options for such disorders is crucial. We previously discovered that a selective mu-opioid receptor (MOR) antagonist, beta-funaltrexamine (β-FNA), inhibited inflammatory signaling *in vitro* in human astroglial cells. Interestingly, the anti-inflammatory properties of β-FNA do not seem to involve actions at the MOR. In a preclinical model, β-FNA was also found to inhibit lipopolysaccharide (LPS)-induced sickness behavior and neuroinflammation in mice when administered immediately post-LPS. In the present study, we explore the extent to which β-FNA is protective when treatment occurs 4 hours after LPS administration. Male C57BL/6J mice were administered LPS (0.83 mg/kg, i.p.) followed by treatment with β-FNA (50 mg/kg, i.p.) immediately or 4h post-LPS, depending on the experiment. Sickness behavior was assessed using a 10-min open-field test, followed by collection of the brain, spleen, and plasma. Levels of inflammatory chemokines/cytokines (interferon γ-induced protein, CXCL10; monocyte chemotactic protein 1, CCL2; and interleukin-6, IL-6) in tissues were measured using an

enzyme-linked immunosorbent assay. β-FNA treatment immediately after LPS administration inhibited LPS-induced CCL2 and CXCL10 (but not IL-6) levels in the brain and plasma at 24h; whereas only CCL2 was inhibited in the spleen. LPS-induced sickness behavior at 24h was also reduced by immediate β-FNA treatment. β-FNA treatment 4h post-LPS was less protective. Inflammation and sickness behavior were more pronounced at 8h than at 24h post-LPS, and β-FNA was overall not protective in the brain or peripheral tissues. One exception was CCL2 expression in the brain at 8h post-LPS, which was inhibited by β-FNA treatment at 4h. The results of this study suggest that the timing of β-FNA treatment is critical for neuroprotection and that β-FNA may have differential central and peripheral effects. Further examination of β-FNA's anti-inflammatory and neuroprotective actions is still necessary.

Disclosures: S. Myers: None. **D. Buck:** None. **K. McCracken:** None. **J.T. Curtis:** None. **R.L. Davis:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.01

Topic: C.08. Ischemia

Title: The impact of exercise prior to stroke in cathepsin B knockout mice

Authors: *K. M. COTTER[1], J. TURCHAN-CHOLEWO[1], M. K. MALONE[1], T. A. UJAS[1], S. J. MESSMER[2], L. J. SCHMIDT[3], A. J. PARKER[3], G. J. BIX[4], A. M. STOWE[1];
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Abstract: Background: LG3 is a peptide derived from the C-terminus of perlecan, a proteoglycan in the vascular extracellular matrix, and plays a neuroprotective/neuroreparative role in the brain after ischemic stroke (PMID: 24323378, 21747167). Crucial to this derivation is cathepsin B, a ubiquitous cysteine myokine protease, which increases in plasma post exercise in both humans and mice. In addition, increased cathepsin B levels contributes to beneficial effects on memory and neurogenesis (PMID: 27345423). Cathepsin B knockout (ctsbKO) mice do not benefit from exercise due to the inability to derive LG3 (PMID: 27345423). **We hypothesize that the neuroprotective benefits of exercise before stroke is lost in ctsbKO mice.**Methods: 3-9 mos.-old ctsbKO male (n=23) and female (n=24) mice were split into sedentary (Sed) and exercise (Ex) cohorts for 3 wks prior to transient middle cerebral artery occlusion (tMCAo), with inclusion criteria &<80% baseline cerebral blood flow (CBF via laser Doppler flowmetry) reduction, returning to <50% baseline CBF at reperfusion. Euthanasia occurred 3 days post-tMCAo. Ex cages contained a monitored running wheel to measure distance run. Submandibular blood was drawn weekly and spleen, blood, and brain collected for flow cytometry (spleen),

histology (brain), and ELISA (plasma). Data were analyzed using FlowJo v10.7.2 Software and stats using GraphPad Prism v9.1.0.Results: ctsbKO mice had significant exclusion based on CBF values with tMCAo in Sed vs Ex cohorts for both sexes (both $p>0.05$), including a high incidence of reperfusion and hemorrhagic transformation. There was no difference in post-stroke neurological deficit score (NDS) between cohorts. All female mice exhibited increases in activated B and CD4 T cells, and innate cells in the spleen. Ex increased monocyte and neutrophils while decreasing macrophages, again only in female mice. There was no correlation to pre-stroke exercise intensity for splenic populations, and male mice did not show differences in post-tMCAo splenic immune cell populations.Conclusion: Sed ctsbKO mice have underlying pathology that precludes successful tMCAo induction similar to other strains previously published (e.g. PMID: 32051245). Exercise had no beneficial effect on the post-stroke NDS of ctsbKO mice, and no peripheral immune effect in male mice. Future studies will confirm infarct volume by histology, and plasma LG3 levels by ELISAs to determine loss of pre-stroke intervention benefit in the absence of cathepsin B.

Disclosures: K.M. Cotter: None. **J. Turchan-Cholewo:** None. **M.K. Malone:** None. **T.A. Ujas:** None. **S.J. Messmer:** None. **L.J. Schmidt:** None. **A.J. Parker:** None. **G.J. Bix:** None. **A.M. Stowe:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.02

Topic: C.08. Ischemia

Title: Transcriptomic hallmarks of ischemia-reperfusion injury

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Abstract: Ischemia reperfusion injury (IRI) is associated with a broad array of life-threatening medical conditions including myocardial infarct, cerebral stroke, and organ transplant. Although the pathobiology and clinical manifestations of IRI are well reviewed by previous publications, changes at the transcriptomic level are extremely lacking. This study aims to reveal a transcriptomic hallmark of IRI using publicly available RNA-sequencing data from 5 experimental organ models. We focused on the acute injury time frame by investigating transcriptional changes up to 48 hours following IRI. We compiled a list of highly reported genes in the current literature that are affected in the context of IRI across various organs and conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of IRI responses both within and across organs. We observed both organ-

specific and shared effects, illustrated by 38 genes that were commonly dysregulated across organ models. Among the shared genes, *Bcl3, Icam1, Itag5, Atf3,* and *Myc* were highly affected, suggesting preserved cellular survival and apoptotic responses to IRI across organs. Specifically, we found up-regulated genes to be involved in cell survival, cell surface signaling, response to oxidative stress, and inflammatory response, while down-regulated genes were predominantly involved in ion transport and synaptic transmission. We also observed specific enrichment of Tumor necrosis factor alpha, Nuclear Factor kappa-light-chain-enhancer of activated B cells, and mitogen-associated protein kinase signaling pathways following IRI across organs. In the final section, we discuss treatment approaches for IRI and their mechanisms of effect by comparing RNA sequencing data from therapeutic interventions with the results of our analyses. This analysis is the first to compare IRI transcriptional changes across organs and provide a comprehensive overview of shared and specific responses. Our findings provide insight on the mechanisms of IRI and offer potential targets for therapeutic interventions.

Disclosures: M. Movahed: None. **S. Brockie:** None. **J. Hong:** None. **M.G. Fehlings:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.03

Topic: C.08. Ischemia

Support: NIH Grant 5R37NS06752S

Title: Role of Poly (ADP-ribose)-dependent Epigenetic Regulation Mediated by ZZZ3 in Ischemic Stroke

Authors: *S.-C. CHOU[1], T.-I. KAM[2], H. PARK[2], V. L. DAWSON[4,3,5,6], T. M. DAWSON[7,3,5,6]; [1]Inst. for Cell Engin., Baltimore, MD; [2]Inst. for Cell Engineering/ Dept. of Neurol., [3]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Inst. Cell Engin., Johns Hopkins Univ. Sch. Med., Baltimore, MD; [5]Adrienne Helis Malvin Med. Res. Fndn., New Orlean, LA; [6]Diana Helis Henry Med. Res. Fndn., New Orlean, LA; [7]Inst. for Cell Engin., Johns Hopkins Univ. Inst. for Cell Engin., Baltimore, MD

Abstract: Role of Poly (ADP-ribose)-dependent Epigenetic Regulation Mediated by ZZZ3 inIschemic StrokeShih-Ching Chou[1*], Tae-In Kam[1, 2], Hyejin Park[1, 2], Valina L. Dawson[1, 2, 3, 4, 5, 6], Ted M.Dawson[1, 2, 4, 5, 61]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA[2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA[3]Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA[4]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine,Baltimore, MD 21205,

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USA**Abstract**Ischemic stroke is a common neurological disorder worldwide, in which the loss ofblood, oxygen, or nutrition supply cause permanent disability or death. Glutamate-mediated excitotoxicity induces neuronal injury following stroke through activation ofpoly (ADP-ribose) (PAR) polymerase-1 (PARP-1) and generation of the death moleculePAR, which has been designated as parthanatos. Previously we identified a cell survivalE3 ligase, Iduna (*Rnf146*) that protects against neuronal injury, we aimed to identifysubstrates that are responsible for neuronal injury. Here we identified the ZZ-type Zincfinger-containing protein 3 (ZZZ3), which is a core regulatory component of Ada-two-A-containing (ATAC) histone acetyltransferase as a potential substrate of Iduna that isinvolved in PAR dependent cell death. Depletion or loss of PAR binding of ZZZ3 showedprotection against parthanatos by disrupting ATAC recruitment to histone H3, leading to asignificant decrease of acetyl-H3K9 after DNA damage. We found that expression ofmacrophage migration inhibitory factor (MIF), a key nuclease in the parthanatos deathcascade, is regulated by PAR binding activity of ZZZ3. Our finding suggesting that PARis involved in epigenetic regulation via ZZZ3-dependent histone modification duringparthanatos. Depletion of ZZZ3 is protective in vivo against middle cerebral arteryocclusion-induced stroke in mice. Inhibition of ZZZ3 could be a potential therapeuticstrategy for the treatment of neurologic disorders where parthanatos plays a pivotal role.

Disclosures: S. Chou: None. **T. Kam:** None. **H. Park:** None. **V.L. Dawson:** None. **T.M. Dawson:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.04

Topic: C.08. Ischemia

Support: PRX18/00241 from the Ministerio de Ciencia, Innovación y Universidades, Spanish Government, BEST/2019/006 from the Conselleria d´Innovació, Universitats, Ciència I Societat Digital, Generalitat Valenciana, Spain to TJ-M
NIH NS100047, AHA Scientist Development Grant 16SDG31500001, NARSAD Young Investigator Grant 25369 and LB692 Nebraska Tobacco Settlement Biomedical Research Development Funds to JYH
NIH NS046742, HD083828, NS100047 and the generous grant from the F.M. Kirby Foundation to RSZ.

Title: Ischemic preconditioning upregulates major molecular players involved in mitophagy

Authors: *T. JOVER-MENGUAL[1,2], H.-R. BYUN[1], B. L. COURT-VAZQUEZ[1], J.-Y. HWANG[3], R. ZUKIN[1];
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Abstract: Transient global ischemia affects millions of people each year and arises as a consequence of cardiac arrest, cardiac surgery, profuse bleeding, near drowning, and carbon monoxide poisoning. Global ischemia in humans or experimentally induced in animals causes selective, delayed death of hippocampal CA1 pyramidal neurons resulting in severe cognitive deficits. To date, there is no known treatment for the neurodegeneration associated with this devastating insult. Loss of mitochondrial function is an early event during cerebral ischemia, ultimately triggering neuronal cell death. Mitochondria are involved in ATP production, ROS generation, inflammation, as well as apoptosis. Selective degradation of damaged or dysfunctional mitochondria through autophagy is known as mitophagy. Ischemic preconditioning is a well-known phenomenon in which a brief, sublethal ischemic insult confers robust neuroprotection to hippocampal CA1 neurons against a subsequent severe ischemic challenge. The molecular mechanisms underlying ischemic tolerance by preconditioning are not fully understood. We recently showed that global ischemia triggers a transient increase in biochemical markers of autophagy, pS317-ULK-1, pS14-Beclin-1, and LC3-II, a decrease in the cargo adaptor p62, and an increase in autophagic flux, a functional readout of autophagy, in selectively vulnerable hippocampal CA1 neurons. However, the role of mitophagy in neuronal death after cerebral ischemia is as yet unknown. Here we show that mitophagy is activated in response to global ischemia in the hippocampal CA1 pyramidal neurons *via* the PINK/PARKIN/p62 signaling pathway together with an increase in phosphorylation at Ser616 of dynamin-related protein-1 (Drp1), a marker for mitochondrial dynamics**.** Additionally, ischemia induces phosphorylation of NFκB and increases expression of the proinflammatory IL-1β in the cytoplasmic fraction. However, no translocation of NFκB to the nucleus was observed. Instead, NFκB increased in the mitochondrial fraction suggesting a role of NFκB signaling in the mitochondria, possibly via p62. We further show that ischemic preconditioning induced an increase of PINK, PARKIN, and BNIP3 expression in the mitochondrial fraction and a decrease of IL-1β expression on the CA1 neurons indicating that mitophagy can be activated by ischemic preconditioning. These findings indicate that activation of the NFκB/p62/mitophagy pathway may be related to mitochondrial dynamics in ischemic preconditioning.

Disclosures: T. Jover-Mengual: None. **H. Byun:** None. **B.L. Court-vazquez:** None. **J. Hwang:** None. **R. Zukin:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.05

Topic: C.08. Ischemia

Title: Effect of estradiol benzoate on the expression of interleukins 6 and 10 in the rat model of acute global cerebral ischemia, determined by optical biosensors based on planar silicon arrays.

Authors: *O. MONTES[1], M. GARCIA[1], O. GONZALEZ[1], R. J. DELGADO[2], G. MORALI[3]; [1]Ctr. de Investigación en Reproducción Animal, Univ. Autónoma de Tlaxcala-CINVESTAV., Tlaxcala, Mexico; [2]Ctr. de Investigación en Biotecnología Aplicada, Inst. Politécnico Nacional., Tlaxcala, Mexico; [3]Inst. Mex. Seguro Social, Mexico City, Mexico

Abstract: The Acute Global Cerebral Ischemia (AGCI) is one of the most important pathologies worldwide in public health due to its high mortality and morbidity rates. The role of molecules such as interleukins (IL) in ischemic patients is known, IL-6, for example, has pro-inflammatory effects and IL-10, on the contrary, decreases the processes of the inflammatory cascade. It has been shown that Estradiol Benzoate (EB) modulates negatively and positively respectively these ILs, however, it is still not known how the concentrations are at different post-ischemia times in rats that have received a pharmacological treatment of (EB). This is important since if it is known, more effective pharmacological treatments could be implemented with the intention of reducing cell damage. In addition to this, our research team search to determine the concentrations of these ILs with a novel spectrometry methodology that will achieve real quantitative measurements of these cytokines, this same technique, already completed, could be used to quantify them interleukins or other molecules of interest in other inflammatory diseases such as SARS-CoV2.
For this study, male rats of the Sprague Dawley strain, young, with on average weights of 350g each have been used. A global ischemic episode was induced in these study subjects, subsequently they were sacrificed at 2,4,6,12 and 24 hours post-ischemia and their blood serum was obtained, the same was measured in a macro-infared spectrophotometer. Finally, we are establishing a new methodology that will allow us to assemble biosensors in flat silicon matrices for the quantification of these ILs (6 and 10) through their interaction with specific antibodies.

Disclosures: O. Montes: None. **M. Garcia:** None. **O. Gonzalez:** None. **R.J. Delgado:** None. **G. Morali:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.06

Topic: C.08. Ischemia

Support: NS088555

Title: The neurotrophic response of B cells to extracellular glutamate following ischemic stroke

Authors: *J. TURCHAN-CHOLEWO[1], V. O. TORRES[3], M. K. MALONE[1], J. F. SIMPSON[1], S. ESTUS[4], E. J. PLAUTZ[5], M. P. GOLDBERG[6], A. M. STOWE[2];
[2]Neurol., [1]Univ. of Kentucky, Lexington, KY; [3]UT SouthWestern, Dallas, TX; [4]Physiol., Sanders-Brown Ctr. Aging, Lexington, KY; [5]Neurol. & Neurotherapeutics, [6]Neurol., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Background: We previously showed that B cells exhibit a cell-specific migration pattern in the post-stroke brain, and post-stroke B cell depletion impedes neurogenesis, increases anxiety, and exacerbates memory deficits in young male mice; deficits mediated by brain regions outside the infarct. N-methyl-D-aspartate (NMDARs) play a critical role in neuronal excitotoxicity after stroke and the presence GluN2A and GluN2B subunits on B cells could be affected by exposure to extracellular glutamate (glu). We hypothesized that the post-stroke microenvironment, including extracellular glu at physiologic levels (i.e. 1µM), can enhance the neurotrophic capacities of B cells to promote recovery. Methods: Splenic B cells were isolated from 3-5 mo-old male uninjured C57Bl/6J mice and 4 days following a 60-min transient middle cerebral artery occlusion (tMCAo; n=6-10/group). B cell NMDAR subunits (GluN2A and Glun2B) were identified by confocal microscopy. B cell viability, NMDARs, and neurotrophin (NT)-related genes were assessed by flow cytometry and qPCR, respectively, in B cells (+/- 5µg/mL LPS) treated with glu (1µM) +/- NMDAR antagonists for 24h. Data were analyzed by BioRad CFX maestro and two-way ANOVA determined in Graphpad Prism.Results: Confocal image analysis revealed that glu treatment decreased surface expression of GluN2A ($p<0.05$), but not GluN2B, in uninjured B cells and increased GluN2A ($p<0.05$) in post-stroke B cells. Stroke also increased GluN2A mRNA, without effect on GluN2B. Furthermore, glu treatment increased NT expression (brain-derived neurotrophic factor (BDNF): 2.28-fold), NT receptor (TrkB: 1.33-fold) and NMDAR subunit (GluN2A: 2.01-fold, GluN2B: 1.27-fold) mRNA expression in LPS-stimulated B cells (vs. untreated controls). LPS treatment increased interleukin (IL)-10 mRNA in B cells independent of glu exposure. Similarly, p75 mRNA level is decreased in LPS-stimulated B cells that was also unaffected by tMCAo or glu treatment. Conclusions: Our data suggest that stroke and exposure to glu alters GluN2A-containing NMDARs on B cells. B cell NMDAR activity is concomitant with upregulation of key NTs and NT receptors. Unlike BDNF, IL-10, a B cell-derived anti-inflammatory cytokine and NT, is not altered by glutamate. These results are the first to identify a mechanism for a glu-induced neurotrophic role for B cells following ischemic injury. Future studies will confirm intracellular protein levels of NTs and NT receptors, as well as determine whether B cell-derived NTs can protect neurons after stroke.

Disclosures: J. Turchan-Cholewo: None. **V.O. Torres:** None. **M.K. Malone:** None. **J.F. Simpson:** None. **S. Estus:** None. **E.J. Plautz:** None. **M.P. Goldberg:** None. **A.M. Stowe:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.07

Topic: C.08. Ischemia

Support: P3E2018
Pro/SNI 2018

Title: Intranasal erythropoietin protects CA1 hippocampal cells, modulated by specific time pattern molecular changes after ischemic damage in rats

Authors: *R. MACIAS-VELEZ, M. RIVERA CERVANTES;
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Abstract: Global cerebral ischemia causes damage to various brain areas; among those vulnerable regions, the hippocampus has shown a selective neuronal death within its parts, particularly Cornu Ammonis 1 (CA1) region is very susceptible to ischemic damage. Erythropoietin (EPO), a multitarget molecule has exhibited neuroprotective properties, especially against cerebral ischemia. However, little effort has been made to determinate both the administration pathway and doses that attenuate neuronal damage. In this study, we investigate the effect on CA1 region of 3 different intranasal doses of recombinant human erythropoietin (rHuEPO) (500, 1000 and 2500 IU/kg) administrated in 3 distinct post-damage temporal schemes (1, 6, and 24 h) against ischemic cellular damage. Furthermore, most effective dose and time were used to evaluate gene and protein expression changes of 3 crucial EPO signaling molecules (EPO, EPOR, and βcR). Under this ischemia model, we observed histopathological damage on CA1-region but rHuEPO administration protected cells against ischemic damage, particularly at 1000 IU dose. Molecular data shows that EPO and its receptor (EPOR) gene expression are upregulated in a short term after damage treatment with rHuEPO (1 h); oppositely, BcR is upregulated in ischemic and Isc + EPO groups in a late scheme. Protein expression data displays no significant changes on EPO expression on evaluated times after treatment, but a tendency to increase 24 h after damage; in the opposite way, EPOR is upregulated significantly 6 h after treatment and this effect last until 24 h. So, our data suggest that a single intranasal dose of rHuEPO (1 h post-injury) provides histological neurorestoration in CA1 hippocampal region, even if we did not observe a dose-dependent effect, the medium dose evaluated (1000 UI/kg of b.w.) was more effective and sufficient to induce molecular changes that provides the initial platform for neuroprotection. Further experiments including βcR protein expression and other related molecules are required to fully understand the protection mechanisms of EPO against ischemia

Disclosures: R. Macias-Velez: None. **M. Rivera Cervantes:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.08

Topic: C.08. Ischemia

Support: NIH RO1 NS099531
NIH RO1 NS101960
NIH RO1 NS109459

Title: Microrna mir-7 availability is essential for promoting the post-ischemic functional recovery

Authors: *S. L. MEHTA[1], A. K. CHOKKALLA[1], S. BATHULA[1], B. KLEAVELAND[2], R. VEMUGANTI[1,3];
[1]Neurolog. Surgery, Univ. of Wisconsin, Madison, WI; [2]Pathology and Lab. Med., Weill Cornell Med., New York, NY; [3]William S. Middleton Mem. Veteran Admin. Hosp., Madison, WI

Abstract: Transient focal ischemia rapidly alters the expression of microRNAs in the brain, including a sustained decrease in the abundance of miR-7a-5p irrespective of age and sex in rodents. We previously showed that miR-7 regulates the levels of α-synuclein (α-Syn), a protein that promotes ischemic brain damage by mitochondrial fragmentation, oxidative stress, and autophagy leading to neuronal death. In addition, we observed that deletion of α-Syn gene curtailed miR-7 mimic-dependent neuroprotection and functional recovery following focal ischemia. In mice, miR-7a and miR-7b (95% homologous) are transcribed from 2 genomic loci. To further understand the active role of miR-7 in the brain, we evaluated the effect of transient middle cerebral artery occlusion (MCAO) in mice that lack both miR-7a and miR-7b (double knockouts; miR-$7^{-/-}$). These mice followed the predicted Mendelian frequency and showed similar sensorimotor and cognitive function to wild-type (miR-$7^{+/+}$) mice measured by the rotarod, beam walk, and Morris water maze tests. However, when subjected to even mild transient MCAO (45 min), miR-$7^{-/-}$ mice showed worsened motor function and significantly increased lesion volume compared to miR-$7^{+/+}$ mice. Furthermore, replenishing miR-7 with a miR-7 mimic (IV injection at 30 min of reperfusion following transient MCAO) restored miR-7 mediated protection and improved motor impairments, potentially through lowering the levels of α-Syn. Our findings suggest that the presence of miR-7 is essential for the recovery of post-stroke brain function.

Disclosures: S.L. Mehta: None. **A.K. Chokkalla:** None. **S. Bathula:** None. **B. Kleaveland:** None. **R. Vemuganti:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.09

Topic: C.08. Ischemia

Support: Nebraska Tobacco Settlement Biomedical Research Development Funds LB692

Title: Inhibition of TREM1-mediated neuroinflammation confers neuroprotection against global ischemia-induced neuronal death.

Authors: *R. URQUHART, T. NILLES-MELCHERT, H. KIM, G. P. JADHAV, J.-Y. HWANG;
Dept. of Pharmacol. and Neurosci., Creighton Univ. Sch. of Med., Omaha, NE

Abstract: Global ischemic stroke is comorbid with cardiac arrest, where loss of cerebral blood flow creates a hypoxic environment in the entire brain. Transient global ischemia drives selective, delayed loss of hippocampal CA1 neurons, contributing to deficits in hippocampal based learning and memory. Elucidating molecular mechanisms underlying global ischemia is essential for development of novel therapeutic strategies to prevent and ameliorate global ischemia induced cell death and cognitive deficits. RNA-seq and Ingenuity Pathway analysis performed on rat hippocampal CA1 following global ischemia induced in vivo via 4-vessel occlusion revealed that within 48HR of ischemic insult expression of triggering receptor expressed on myeloid cells-1 (TREM1) was elevated. TREM1 is a pivotal innate immune receptor that initiates and amplifies neuroinflammation via synergism with Toll like immune receptors. Thus, it was hypothesized that global ischemia promotes TREM1-mediated neuroinflammation, and inhibition of TREM1 can prevent neuroinflammatory signaling and attenuate global ischemia-induced cell death and cognitive deficit. Here we show that hippocampal CA1 TREM1 expression was evaluated following global ischemia, revealing that mRNA and protein levels were increased within 24 and 48HR respectively. We further show that immunolabelling of hippocampal CA1 tissue revealed that TREM1 was localized in CD31+ positive cells, but not Iba1+, NeuN+, or GFAP+ cells, suggesting that TREM1 mediated neuroinflammation is initiated by CD31+ immune cells trafficked into the brain following ischemic insult. Additional immunolabelling is being performed to identify the cell type responsible for TREM1 action. We tested TREM1 inhibitors to determine if TREM1 inhibition can rescue global ischemia-induced cell death and cognitive deficit. We are currently collecting the data assessing the ability of TREM1 inhibition to rescue neurons and cognitive deficit via hippocampal-based learning and memory tests. Our results will document a causal role for TREM1- mediated neuroinflammatory signaling in global ischemia-induced neuronal death. This research illuminates the molecular mechanisms underpinning TREM-1 mediated neuroinflammation and advances approaches for treating global ischemia.

Disclosures: R. Urquhart: None. **T. Nilles-Melchert:** None. **H. Kim:** None. **G.P. Jadhav:** None. **J. Hwang:** None.

Poster

P324. Molecular Mechanisms of Ischemia

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P324.10

Topic: C.08. Ischemia

Support: American Heart Association (16SDG30320001)
LSUHSCS, Center for Cardiovascular Diseases and Sciences (110101107A)

Title: Inflammatory responses provide critical support for neurogenesis and functional recovery following cerebral ischemia

Authors: D. R. HERNANDEZ[1], P. M. BOUDREAUX[1], K. C. SIMPSON[1], *K. M. RODGERS[2];
[1]Cell. Biol. and Anat., [2]Cell. Biol. and Anatomy, Neurol., LSU Hlth. Shreveport, Shreveport, LA

Abstract: Introduction: Replacement of dead neurons following ischemia, either via enhanced endogenous neurogenesis or stem cell therapy, has been highly sought, but the low survival rate of newly generated neurons has left doubt about the therapeutic potential of adult neurogenesis. However, stroke in the young injured brain reveals a greater degree of plasticity and capacity for repair, along with enhanced post-ischemic functional outcomes compared to the adult. Our findings suggest that a possible mechanism is the immune response in the acute phase of stroke, which has a powerful age-related influence on neurogenesis and functional recovery. Our preliminary studies reveal an anti-inflammatory microglial signature during the acute phase of stroke in juveniles and proinflammatory in adults. While microglial responses have been shown to be both neuroprotective and neurodegenerative following brain injury, it has been generally accepted that activation of microglia during the acute response to stroke is detrimental for neurogenesis and neuronal replacement. However, our findings indicate that early microglial responses are key to survival of newborn neurons in juveniles.
Objective: We utilized a juvenile stroke model to study the paracrine signaling triggered by ischemia in activated microglia and its consequence on newborn neuron survival and post-ischemic functional outcomes.
Methods: Electrocorticography (ECoG) was recorded from cortical surface electrodes and striatal depth electrodes implanted following transient middle cerebral artery occlusion (MCAO). Microglia were attenuated with Ibudilast (10 mg/kg), a glial cell activation inhibitor. Neurogenesis and inflammatory markers were examined and functional outcomes assessed with neurobehavioral measures and ECoG recordings.
Results: Short-term inhibition (4d) of microglial activation during the acute phase after stroke resulted in reduced neurogenesis and reversal of motor recovery and neuroplasticity in MCAO-injured juveniles. In contrast, treatment in MCAO-injured adults improved neurogenesis and

functional outcomes revealing age-related differences in post-ischemic immune responses. **Conclusions:** These striking findings provide evidence of a supportive role for microglia in neurogenesis and improved functional recovery following stroke in juveniles. This novel insight into early, anti-inflammatory microglial responses after stroke may alter strategies for effective stroke treatment, promoting substantial post-ischemic neuronal replacement and functional recovery in both juveniles and adults.

Disclosures: D.R. Hernandez: None. **P.M. Boudreaux:** None. **K.C. Simpson:** None. **K.M. Rodgers:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.01

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS107451

Title: Spatial transcriptomics exploration of the brain-electrode interface

Authors: *Q. A. WHITSITT[1], B. K. PATEL[2], B. HUNT[1], E. K. PURCELL[2];
[1]Biomed. Engin., [2]Michigan State Univ., East Lansing, MI

Abstract: The foreign body response to implanted electrodes in the brain has been identified as an important area of research for the future development of brain-machine interfaces and in vivo electrophysiology. Due to many mechanisms such as reactive astrogliosis and neuron cell death, the functional makeup of tissue immediately surrounding the electrode after implantation is significantly altered from its original state. To discover possible functional changes affecting device function and longevity, spatial transcriptomics was implemented to identify relevant clusters of differential gene expression driven by electrode implantation. This RNA-sequencing technique (10x Genomics, Visium) uses spatially coded, RNA-binding oligonucleotides on a microscope slide which, after cDNA synthesis, are used to spatially identify each sequencing read. For these experiments, sections of rat motor cortex implanted with Michigan-style silicon electrodes were mounted on the Visium slide for processing. Each tissue section was labeled for neuronal nuclei (NeuN) and astrocytes (glial fibrillary acidic protein, GFAP) using immunohistochemistry to provide a spatial reference for mapping each sequencing read relative to the device tract. Preliminary results from rat motor cortex at 1 week and 6 weeks post implantation have shown up to 886 differentially expressed genes within 350μm of the device tract compared to tissue greater than 500μm from the device tract. Many of these genes are related to biological mechanisms previously reported in studies of the foreign body response to

implanted electrodes, while others are novel to this study. These results will provide a foundation for future reprograming approaches to both improve and measure the effects of gene expression on the long-term stability of recording from implanted electrodes in the brain. Ongoing work will include additional timepoints and more samples per timepoint as we gain a better understanding of the dynamic changes taking place in the brain at a cellular level in response to electrode implantation.

Disclosures: Q.A. Whitsitt: None. **B.K. Patel:** None. **B. Hunt:** None. **E.K. Purcell:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.02

Topic: E.05. Brain-Machine Interface

Support: F99NS118738
R01NS107451

Title: Gene Expression Changes and Adhesion in Cultured Reactive Rat Astrocyte Models in Comparison to Device-Associated Effects in the Brain

Authors: *T. RIGGINS[1], E. HUNTER[2], B. HUNT[1], E. K. PURCELL[1];
[1]Biomed. Engin., [2]Computer Sci., Michigan State Univ., East Lansing, MI

Abstract: Intracortical electrode arrays have great promise to treat many neurodegenerative diseases. However, device performance can decrease sharply post-implantation, where one report suggests that over half of those failures are attributed to meningeal and astroglial encapsulation around the probe. To address this, many researchers have moved to making softer, smaller implants, in hopes of minimizing the mechanical mismatch between the brain and the device interface. In parallel, new observations suggest that reactive astrocytes encompass a spectrum of functional states, which can be partially delineated by assessing differences in their gene expression. The purpose of our studies is to develop *in vitro* models of reactive astrocytes that can be used to understand changes in gene expression surrounding devices in the intact brain as well as the interaction with specific features of electrodes. Using a working tissue culture model of inflammation, we are directly measuring device surface roughness and correlating it to astroglial activity. To understand the impact of surface roughness on how astrocytes encapsulate probes, atomic force microscopy technique offers a venue to image topological probe features, measure nanoscale roughness and bending stiffness. These measurements are being used to correlate with astroglial cellular adhesion to probe materials, determined with immunohistochemistry methods. In addition, we are using RNA-sequencing to compare changes

in genetic expression in our reactive models to that of intact brain, and to that of rat tissue harvested from implanted probes. This cross comparative study will be used to improve our understanding of the functional identity of reactive astrocytes surrounding devices and determine an optimal surface roughness range that may reduce cell adhesion and indirectly support device longevity. This new information could inform potential guidelines for designing intracortical electrode arrays.

Disclosures: T. Riggins: None. **E. Hunter:** None. **B. Hunt:** None. **E.K. Purcell:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.03

Topic: E.05. Brain-Machine Interface

Support: NSF CAREER #1943716

Title: Spatiotemporal Protein Expression Patterns of RNA-Sequencing Identified Genes Around Implanted Silicon Electrode Arrays

Authors: *C. H. THOMPSON, N. HEELAN, E. K. PURCELL;
Biomed. Engin., Michigan State Univ., East Lansing, MI

Abstract: Implanted neural interfaces enable numerous clinical and research applications but are often hindered by long-term signal instability. The tissue response of the brain to implanted device is a suspected contributor to inconsistent performance. The tissue response has been traditionally characterized by inflammation, glial scaring, and neuronal cell death. New RNA-sequencing data from our lab suggests that mechanisms of the tissue response are more complex than previously anticipated and that broad spatiotemporal changes in gene expression drive the biological response to implanted devices. We identified numerous differentially expressed (DE) genes associated with synaptic function (Bsn, Arc), myelin formation (Plp1, Mbp, Tf), and cellular identity (Ptbp1) which have significant biological implications in neurodegenerative disease and traumatic brain injury but are novel in the context of implanted cortical devices. However, the mRNA-to-protein expression relationships, protein localization, and possible functional changes associated with the DE of these genes are not yet well understood. To provide greater understanding of how these genes influence the tissue response to implanted electrodes we implanted Michigan style probes into motor cortex of male Sprague Dawley rats to evaluate tissue response at 24 hours, 1 week, and 6 weeks post-implantation (n=6 per timepoint). By utilizing immunofluorescence and quantitative fluorescence intensity analysis we have systematically evaluated the spatiotemporal expression and cell-type specific localization of

these proteins relative to naïve brain tissue. The results of this study suggest that in many cases, the protein expression of these genes mirror the spatiotemporal patterns of gene expression identified by our previous RNA-sequencing results. Importantly, we were able to identify the cellular localization and specific cell types primarily expressing these proteins of interest. This study expands upon previous understandings of the tissue response to implanted devices while also providing evidence for the dynamics of these proteins and their role in tissue response.

Disclosures: C.H. Thompson: None. **N. Heelan:** None. **E.K. Purcell:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.04

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neurosciences Institute
NSF GRFP DGE-1656518

Title: Synchronization of wireless electrophysiology and markerless kinematics for large, unconstrained animals

Authors: *M. P. SILVERNAGEL[1], A. L. LING[1], P. NUYUJUKIAN[2,3,1,4,5];
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Abstract: Motor systems neuroscience studies in large animals typically constrain body movement from regions not under study, thus minimizing the presence of confounding variables. This promotes consistent, repeatable behaviors, providing robust datasets that have fueled neuroscience studies for decades. The downside to this approach is that the observed neural data may differ between constrained and more naturalistic, unconstrained movements. Utilizing a markerless motion tracking and kinematic extraction platform developed by our group, we present the first recordings of wireless electrophysiology synchronized to full-body kinematics. Recordings were obtained from 96 channels in both premotor (PMd) and motor (M1) regions of an adult, male rhesus macaque. Sessions took place in a large, 2.1 x 2.1 x 2.4 m (l x w x h) enclosure and lasted 10 to 20 minutes. The animal then performed a sterotyped walking task or engaged in unstructured, neuroethologically relevant behaviors, including foraging, object interaction, and hunting for insects. By sending data acquisition signals used by the motion tracking platform to the commercial wireless neural data acquisition system, kinematic and neural data were aligned within 50 us. With this synchronized neural and kinematic data--which contains movement tasks that have not been previously explored by the motor systems

neuroscience community--we look to generate novel insights into the functioning of the mammalian motor system.

Disclosures: M.P. Silvernagel: None. **A.L. Ling:** None. **P. Nuyujukian:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.05

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neurosciences Institute
NSF GRFP DGE – 1656518
Stanford School of Medicine's Dean's Postdoctoral Fellowship
American Heart Association Predoctoral Fellowship - 828653

Title: A neuroelectrophysiology-compatible platform for lesioning through a microelectrode array

Authors: *I. E. BRAY[1], S. E. CLARKE[2], P. NUYUJUKIAN[2,3,1,4,5];
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Abstract: Lesion studies have established causal connections between brain and behavior, providing fundamental insights in medicine and neuroscience. Intracortical neuroelectrophysiology from microelectrode arrays, a technique that has transformed motor systems neuroscience, could further impact our causal understanding of the brain if seamlessly integrated with lesioning. Although many lesioning methods exist, most are not compatible with stable neuroelectrophysiology pre- and post-lesion. Here we present a platform for creating precise electrolytic lesions through chronically implanted microelectrode arrays without compromising neuroelectrophysiological recordings. We create the lesion by applying direct current through two electrodes of the microelectrode array. A custom-built current source provides stable current in the face of variable electrode impedance and allows for controlled, repeatable lesions in awake-behaving animals. The choice of the electrode pair and the duration and intensity of the current affects the location and the extent of the lesion. This platform was validated through testing in multiple species. *Ex vivo* and *in vivo* lesions through Utah electrode arrays in sheep and pigs (eleven *ex vivo* and five *in vivo*, with a total of 61 lesions) were performed to validate device performance and calibrate parameters. Histology from a lesion (150µA of DC current for 45 seconds) in one adult male rhesus macaque, shows an ablated core surrounded by rarefied tissue. These parameters were used for fourteen subsequent in vivo

lesions in two awake-behaving adult male rhesus macaques. Variable voltage traces and stable current traces during this *in vivo* lesioning demonstrate that the feedback circuit operates as intended. Measurements of spiking activity before and after lesioning confirm that this approach is compatible with neuroelectrophysiology. In summary, this electrolytic lesioning method avoids disruptive procedures, provides sub-millimeter control over the extent and location of the injury in cortex, and permits neuroelectrophysiological recording of single-unit activity from the same neuronal population after lesioning. The electrolytic lesioning technique presented here results in a behavioral deficit during reaching, seen in the two awake-behaving adult male rhesus macaques, facilitating causal investigations of the recorded neuronal population's role in generating behavior. As the animals behaviorally recover, this technique simultaneously provides insight into local reorganization after injury.

Disclosures: I.E. Bray: None. **S.E. Clarke:** None. **P. Nuyujukian:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.06

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neurosciences Institute
Stanford School of Medicine's Dean's Postdoctoral Fellowship
NSF GRFP DGE – 1656518
American Heart Association Predoctoral Fellowship - 828653

Title: Electrolytic lesion with microelectrode arrays

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Abstract: Background: Emerging brain interfacing technologies hold promise for treating disease throughclosed-loop neuromodulation. However, a major scientific knowledge gap exists between the recordedactivity of large neuronal populations and their related function. This disconnect prevents identificationof the mechanisms necessary to restore a damaged network, prompting calls from the US NationalInstitutes of Health for primate models of brain injury that advance the translation of basicneuroscience findings into viable clinical therapies. **Methods**: Electrolytic lesions are performed in awake-behaving adult male rhesus macaques throughchronically implanted Utah arrays in motor cortex. This approach allows for otherwise undisturbedneuron population recordings before, and immediately after spatially precise damage

that targets theneuronal correlates of a standard reaching task. Thousands of reaches are performed every 24 hoursduring electrophysiological recordings, providing ample statistical power to track the time course ofrecovery. **Results**: Histology indicates a lesion volume of approximately 1.77 mm^3, consisting of an ablated coreand partially damaged tissue reminiscent of human brain injury. A data set of 14 lesions has beengathered from two animals demonstrating changes in single neuron activity and population structurethat accompany slower, less accurate reaches. Specifically, we observe a strong correlation between theextent of behavioral deficit and changes in the variance explained by the top five principal componentsof neuron population spiking activity. During behavioral recovery, local reorganization of the damagednetwork is recorded by the microelectrode array. **Conclusion**: By seamlessly integrating lesions with existing recording capabilities, we present aunique opportunity to study causal cortical dynamics and neuronal circuit reorganization usingchronically implanted microelectrode arrays.

Disclosures: S.E. Clarke: None. **I.E. Bray:** None. **P. Nuyujukian:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.07

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neuroscience Institute
NSF GRFP DGE-1656518

Title: Markerless pose tracking for task-based and free behavior

Authors: *A. LING[1], M. P. SILVERNAGEL[1], P. NUYUJUKIAN[2,3,1,4,5];
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Abstract: Studies of unconstrained and ambulatory behavior are necessary to observe the full richness of neural activity associated with movement. However, no platforms exist to capture full-body markerless natural macaque behavior synchronized with neural data in a large space. We present the first markerless, full-body motion tracking and synchronized wireless neural electrophysiology platform for large, ambulatory animals, consisting of four RGB-D cameras that provide a 360 degree view of a 4.5m^2 observational enclosure. With this platform, a wide range of freely moving and naturalistic full body kinematics can be extracted providing novel insights into the functioning of the mammalian motor system. A large observational enclosure, measuring 2.1x2.1x2.4 meters, was outfitted with four RGB-D cameras. High resolution depth

data acquired on a single Linux desktop. Point clouds were generated for each camera by converting every depth pixel to a 3D coordinate space, and resulted in a full volumetric point cloud created by aligning point clouds from each camera to a single reference frame. Three dimensional full body kinematics were extracted from the point cloud data using an articulated skeletal model and an optimization algorithm for one macaque. The skeleton was created in a bottom-up fashion in Blender from primitive meshes made in MeshLab. Bayesian Optimization maximized the number of points inside each segment to return the best fit joint angle and location. Full body kinematics were extracted for a variety of behaviors ranging from task-based to unprompted, naturalistic behaviors. Multiple kinematic trials were captured of the gait cycle and arm reaching during a freely moving walking task, two behaviors we will further explore with neural data. Additionally, kinematics were extracted for predatory behavior and interactions with large objects, naturalistic behaviors that are challenging to capture in a constrained space. Kinematics for a macaque were extracted for 150 frames as he walked over a large tire to forage in its center and 36 frames as he swiftly attempted to catch a fly, highlighting the platform's ability to capture dexterous movements. To validate the performance of the algorithm, error was calculated from 300 frames of the walking task. The mean Euclidean distance in cm between the algorithm's and the hand-labelled position summarized the error was between 3-5 cm, surpassing other 3D markerless platforms' accuracy. Subsequent effort has led to neural synchronization of the kinematics to wireless electrophysiology.

Disclosures: A. Ling: None. **M.P. Silvernagel:** None.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.08

Topic: E.05. Brain-Machine Interface

Support: Defense Advanced Research Projects Agency (grant number HR00111990043).

Title: Long-term intracortical microelectrode array performance in a human: a five-year retrospective analysis

Authors: S. COLACHIS[1], C. DUNLAP[3], *D. FRIEDENBERG[2];
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Abstract: Microelectrode arrays (MEAs) used to record neural signals for intracortical brain-computer interfaces (BCIs) are subject to biological, material, and mechanical disruptions that degrade BCI performance. While these MEA disruptions have been well-studied in non-human primates, less is known about long-term signal quality in humans due to a limited number of

chronic implantations. Our group has collected one of the largest neural recording datasets from a Utah MEA in a human subject, spanning over five years (2014-2019). We analyzed signal quality and BCI performance across 448 clinical recording sessions during this period. Signal quality was assessed for each session using metrics including signal-to-noise ratio, spike peak-to-peak voltages, channel correlation, electrode impedance, and others. Signal quality decreased rapidly in the first year, then more gradually through the remainder of the study. Despite this signal degradation, BCI decoding accuracy in a four-movement motor imagery task remained stable throughout the study. We also identified several acute signal disruptions that affected signal quality on the time scale of hours to days. We characterized the signal effects of these disruptions then utilized this information to hypothesize or identify the disruptions' root cause. Drawing support from our analyses, we discuss potential identification and mitigation strategies for BCI signal disruptions. Our work addresses the knowledge gap around long-term MEA performance in humans, providing longevity and efficacy data points to better describe the performance of implantable neural sensors in a human population. Furthermore, characterizing both chronic signal trends and common signal disruptions will support researchers in developing automated countermeasures to maintain or rescue BCI performance following unexpected disruption events. The trial was registered on ClinicalTrials.gov (Identifier NCT01997125) and conformed to institutional requirements for the conduct of human subjects research.

Disclosures: S. Colachis: A. Employment/Salary (full or part-time):; Battelle. **C. Dunlap:** A. Employment/Salary (full or part-time):; battelle. **D. Friedenberg:** A. Employment/Salary (full or part-time):; battelle.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.09

Topic: E.05. Brain-Machine Interface

Support: NeuroOne Medical Technologies

Title: Device testing and initial clinical use of thin film electrodes for intracranial recording and monitoring of brain activity

Authors: *A. KULLMANN, S. MERTENS, M. CHRISTIANSON, D. ROSA, F. KULLMANN, C. DIAZ BOTIA;
NeuroOne Med. Technologies Corp., Eden Praire, MN

Abstract: Introduction. Invasive intracranial recordings are performed as part of pre-surgical work-up in patients suffering from drug resistant epilepsy (DRE). Strips and grids electrodes are

placed in the subdural space for several days and data are used to identify the extent of the epileptic region. Although these electrodes have been in the US market for decades, their fabrication, materials and properties have hardly changed. They are silicone based, thick (>0.5mm), manufactured by hand, and do not optimally conform to brain convolutions. Technological solutions that overcome these limitations are needed. **Objective.** Here we describe a battery of electrochemical, electrical and biocompatibility tests of new thin film electrode for use in clinical practice. We also present preliminary post-market user feedback data from initial clinical use. **Methods.** Thin film electrode (0.08mm) arrays were manufactured from polyimide with platinum contacts (2-16 contacts, 3 mm contact diameter, spaced 10 mm apart). Electrochemical and electrical testing included electrolyte interface impedance, cyclic voltammetry and other impedance and isolation tests for proving device safety and performance. Biocompatibility was assessed in in vitro and in vivo tests. Post-market data were collected employing a user feedback questionnaire using a rating scale (1 to 5, with 5 being the highest).**Results.** Thin film electrodes met all electrochemical, electrical and biocompatibility testing required for FDA clearance (obtained Nov 2019). Electrodes (n=19) were used in clinical practice between Nov 23, 2020 and May 3, 2021 in the epilepsy monitoring units and for intraoperative monitoring (IOM) for recording brain activity in (a) DRE patients undergoing evaluation for potential surgical resection, and (b) patients undergoing tumor resection. Neurosurgeons (users; n=6) rated the electrodes between 3 and 5 in regard to their properties (thickness, flexibility, weight), features (ease of placement, conformability to the brain areas) and performance (qualitative signal recording quality) during surgical implantation.**Conclusions.** These results demonstrate safety and performance of the first thin film electrode technology cleared by the FDA for use in clinical practice. Initial clinical use indicated that the technology is feasible for surgical implantation with greater flexibility compared to silicone based cortical electrodes. This technology has the potential to minimize invasiveness of subdural recordings and improve surgical outcomes.

Disclosures: A. Kullmann: A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne. **S. Mertens:** A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne. **M. Christianson:** A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne. **D. Rosa:** A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne. **F. Kullmann:** A. Employment/Salary (full or part-time):; full time employee. **C. Diaz Botia:** A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne.

Poster

P545. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P545.10

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS F99NS124186
NIH NINDS R21NS108098
NIH NINDS R01NS094396

Title: Two-photon visualization of novel CX3cr1+/Cspg4+ reactive cell population around chronic intracortical neural electrode interfaces

Authors: *S. M. WELLMAN[1,2], M. M. DOUGLAS[1], T. D. Y. KOZAI[1,2,3,4,5];
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Abstract: Development of advanced neural interfacing technology is accelerating our understanding of nervous system health and disease at a rapid pace. Yet, despite their potential as tools for neuroscience discovery and clinical rehabilitation, intracortical electrodes regularly suffer from depreciations in detecting and modulating brain activity as a result of severe biological responses to a foreign body. Due to the inherent complexity of the brain's immune response to device injury, the mechanisms which govern biological failure modes of neural implants are currently unknown. Efforts to pinpoint exact biological correlates of device performance are confounded by a poorly understood heterogeneity of immune cells (i.e. myeloid cells) within the brain which are known to regulate tissue homeostasis, inflammation, and wound repair following injury. To elucidate the spatiotemporal behavior and fate of different myeloid populations within the brain after device implantation, we employed two-photon imaging of a dual fluorescent CX3cr1-GFP and Cspg4-DsRed mouse model around chronically implanted intracortical electrode arrays. With this model, we have identified a distinct population of dynamic cells around implanted devices separate from GFP+ microglia and DsRed+ glia and pericyte populations. These cells simultaneously express GFP+ and DsRed+ fluorophores denoting constitutive expression of both *CX3cr1* and *Cspg4* promoters. The emergence of dual-labeled CX3cr1+/Cspg4+ cells began 3-4 days following implantation and appeared in close spatial proximity (<100 μm) to the implanted probes. Additionally, these cells demonstrated active process extension and retraction and soma motility both in tissue regions adjacent to the implant and on the device surface itself within the initial weeks post-implantation. Whereas device coverage of individual CX3cr1+ or Cspg4+ cells have been previously recognized, here we report spatially and temporally distinct surface encapsulation by CX3cr1+/Cspg4+ cells, suggesting a potential role as a novel reactive cellular player around chronically implanted microelectrodes. Furthermore, dual-labeled CX3cr1+/Cspg4+ cells were not observed within

contralateral (unimplanted) brain regions, suggesting an injury-specific emergence and response to chronically implanted devices. Future work further characterizing the different heterogenous profiles of myeloid lineage cells and their pathological roles within the brain will facilitate the development of more biologically adaptive implants and engineer innovative therapeutic strategies to combat the body's inherent immune response to brain injury and disease.

Disclosures: S.M. Wellman: None. **M.M. Douglas:** None. **T.D.Y. Kozai:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.01

Topic: F.01. Neuroethology

Support: SSAR Roger Conant Award
Herpetologist League E.E. Williams Grant
SICB Grant in Aid of Research
Chicago Herpetological Society Graduate Grant in Herpetology
Animal Behavior Society Grant in Aid of Research

Title: Reproductive State Modulates Visual Behavior and Retinal Spectral Tuning in the Green Treefrog

Authors: *W. G. WALKOWSKI[1], A. SANTANA[2], T. GASTON[2], W. C. GORDON[2], N. G. BAZAN[2], H. E. FARRIS[1];
[1]Cell Biol. & Anatomy; Neurosci. Ctr. of Excellence, [2]Neurosci. Ctr. of Excellence, Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Hormones are known to affect CNS circuitry mediating behavioral responses to stimuli. Their effect on sensory receptor organs is less understood. This study focused on hormonal modulation of the retina and its consequences on the reproductive behavior of the green treefrog (*Hyla cinerea*). We examined the effects of reproductive status on retinal spectral sensitivity, or the ability to detect different wavelengths associated with visual signals. **Our hypothesis was that hormones modulate stimulus sensitivity in the retina during reproductively receptive phases, specifically influencing wavelength processing important to mate choice behavior**. *H. cinerea* are sensitive to wavelengths spanning the human visible light spectrum and beyond. Experiments used electroretinograms (ERGs) to compare stimulus threshold and response amplitude in the retina of reproductive and non-reproductive frogs. Our findings indicated that female reproductive state was correlated with an increase in spectral sensitivity and a broadening of spectral tuning. However, sensitivity to 530-570nm of light remained unchanged. Spectrometer measurements of the frogs' dorsal surface indicated that peak

reflectance is 540nm, suggesting sensitivity to dorsal coloration does not play a role in reproductive signaling. We propose that modulation of retinal tuning increases sensitivity to a lateral white stripe, which has broad spectral reflectance. Ongoing behavioral trials will test this hypothesis. Preliminary results suggest that modulation of relative wavelength sensitivity of the retina could function to alter visual responses to body parts with broadband reflectance, suggesting endocrine control of reproductive communication.

Disclosures: W.G. Walkowski: None. **A. Santana:** None. **T. Gaston:** None. **W.C. Gordon:** None. **N.G. Bazan:** None. **H.E. Farris:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.02

Topic: F.01. Neuroethology

Support: National Science Foundation[CAREER #1551526]

Title: Determining saccade-like turn refinement during the establishment of visually guided routes in Pogonomyrmex occidentalis

Authors: *J. DE LA PENA[1], T. R. LANGDON[1], E. CORREA[2], V. CO[1], L. ALVAREZ VALENCIA[1], D. D. LENT[1];
[1]Dept. of Biol., [2]Dept. of Mechanical Engin., California State University- Fresno, Fresno, CA

Abstract: Determining saccade-like turn refinement during the establishment of visually guided routes in *Pogonomyrmex occidentalis*
Authors: Joanna P de la Pena, Taylor R Langdon, Edward Correa, Vince Co, Leslye Alvarez Valencia, David D Lent
This research aims to better understand how animals learn to navigate in natural and complex environments. Ants are an ideal model to study navigational behavior and several species have been shown to utilize visual features and other sensory information in their environment to drive a range of navigational behaviors. Here we describe the early visual navigation behavior of the western harvester ant (*Pogonomyrmex occidentalis*). During foraging, the ants produce saccade-like turns (SLTs) towards learned features to maintain trajectory. This behavior allows the ant to sample the visual environment intermittently and correct their routes after encountering an obstacle or other disruptions. It has been shown that once SLTs have been established ants will reliably produce the corrective turns to stay on route, but how these SLTs develop and are refined during early learning is poorly understood. Our research investigated the development and refinement of SLTs in early route establishment within a naturalistic environment. The experiments were done in the laboratory using a high-resolution 360-degree naturalistic

panoramic environment. Ants were filmed during their learning walks and the direction and number of SLTs were recorded. We determined ants produce a greater number but less directed SLTs in their early foraging routes. As learning progresses, SLT production decreased and became more refined towards features and the goal. We identified certain visual features within the panorama that drive these SLTs, resulting in ants directing their orientation and foraging behavior to reach their goal. With the visual cues used by individual ants identified, we characterized the similarities and differences in strategies employed by ants to develop robust visually guided routes. Our data has provided novel insight into how different visual cues become the primary guidance stimulus, but we need to further investigate which individual cues or features are necessary and sufficient for visual guidance. This research, thus far, has provided us with a better understanding of the early behavioral mechanisms employed by ants in natural environments for robust navigation.

Disclosures: J. de la Pena: None. **T.R. Langdon:** None. **E. Correa:** None. **V. Co:** None. **L. Alvarez Valencia:** None. **D.D. Lent:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.03

Topic: F.01. Neuroethology

Support: NSF Grant 1931154

Title: Examining potential memory formation in cnidarians: a non-associative learning assay induces changes in mechanoreceptive behavior in two species of cnidarians, Nematostella vectensis and Hydra littoralis.

Authors: *A. N. TAMVACAKIS, N. NAKANISHI;
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Abstract: Does learning and memory formation occur in cnidarians, animals whichlack centralized nervous systems? Sensitization is a form of non-associativelearning in which a repeated stimulus results in enhanced behavioral responsesto related stimuli. Sensitization is hypothesized to be an evolutionarily earlyform of neuronal plasticity and memory formation. However, it has beenprimarily studied in bilaterians, animals with relatively organized,centralized nervous systems. Cnidaria (sea anemones, hydrazoans, jellyfish, and others) is thesister-taxon to Bilateria, and its members possess diffuse interconnected nervenets rather than centralized ganglia. Cnidarians exhibit simple, tractablebehaviors. One common behavior is body contraction in response to weak tentacletouch. We developed a nociceptive sensitization assay to determine whethercnidarian touch response is enhanced following a series

of noxious trainingstimuli. We examined changes in response to tentacle touch following nociceptivesensitization training in two species of cnidarians, the sea anemone *Nematostellavectensis* and the hydrazoan *Hydra littoralis*. Baseline bodycontraction times in response to tentacle touch averaged 54.01 seconds for *Nematostella*and 41.94 seconds for *Hydra*. Following sensitization training,both species significantly increased the amount of time in which their bodycolumns remained contracted in response to touch, while handled control animalsexhibited no difference from their baseline contraction times. The increasedcontraction time was observed at 18 and 24 hours following the trainingsession, but returned to pre-test levels by 48 hours after training. The enhanced response to tentacle touch following training resembledpreviously described changes in behavior occurring with nociceptivesensitization in bilaterian species. While more must be done to understand thepotential neurological and genetic underpinnings of this sensitization-likeresponse, this study provides a testing framework that can allow researchers todetermine whether a form of non-associative learning could occur in animalswith diffuse nervous systems. By identifying tractablemodels of learning-like behavior adaptations in species outside Bilateria, wecan better understand how learning evolved, and whether there are shared neuralmechanisms underlying learning across groups of animals with contrastingnervous system architectures.

Disclosures: A.N. Tamvacakis: None. **N. Nakanishi:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.04

Topic: F.01. Neuroethology

Support: NSF Grant 1846520

Title: Can the Acoustic Parasitoid Fly Ormia ochracea Eavesdrop and Respond to Novel Cricket Songs?

Authors: *I. ABDULKARIM[1], M. CARLSON[2], J. H. GALLAGHER[3], A. W. WIKLE[3], R. M. TINGHITELLA[3], N. LEE[1];
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Abstract: The acoustic parasitoid fly Ormia ochracea eavesdrops on male cricket calling songs to locate appropriate host crickets for the development of their larval young. As parasitism by O. ochracea results in almost certain cricket death, the flies act as a strong source of selection against song production in host cricket species. In response to this selection pressure, some Hawaiian populations of Teleogryllus oceanicus have evolved changes in wing morphology resulting in a novel “purring” song type. The temporal and frequency characteristics of the new

song type differ from those of the ancestral calling song. Hawaiian O. ochracea are exposed to the new song type with a lower sound intensity (purrs= 53 dB SPL, ancestral= 70 dBA at a distance of one meter), while continental populations of O. ochracea are not. In the present study, we test the hypothesis that if Hawaiian O. ochracea have evolved to detect and respond to novel cricket songs, then detection thresholds across varying song characteristics, such as carrier frequency, should be lower than other populations, a story which has yet to be told.We presented Floridian and Hawaiian O. ochracea with synthetically generated cricket songs varying in carrier frequency and sound intensity. Behavioral response thresholds were evaluated using a spherical treadmill system, and excitatory neural response maps were generated from summed local field potential recordings from the cervical neck connective. Both populations exhibited response thresholds that were sharply tuned to 5 kHz, which matches the dominant frequency of ancestral cricket songs. While response thresholds were lowest at 5 kHz for both populations (FL: 32.22dB±7.36, HI: 36.53dB±11.17, mean±SD), Hawaiian flies exhibited significantly higher response thresholds at higher sound frequencies as compared to Floridian flies. Floridian and Hawaiian flies exhibited similar neural response thresholds, which were approximately 5-10 dB SPL lower than behavioral thresholds. Our findings suggest that the behavioral and neural response thresholds of Floridian O. ochracea are at lower intensities than those of Hawaiian populations, and that there are significant differences between the abilities of geographically separated populations of O. ochracea to detect and respond to novel cricket songs.

Disclosures: I. Abdulkarim: None. **M. Carlson:** None. **J.H. Gallagher:** None. **A.W. Wikle:** None. **R.M. Tinghitella:** None. **N. Lee:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.05

Topic: F.01. Neuroethology

Support: NIH BRAIN Initiative U01NS108637

Title: Characterization of a rapid defensive behavior in a recently introduced model gastropod

Authors: *O. H. BERG[1], A. BOUTKO[2], V. K. MISTRY[3], W. N. FROST[2], J. W. BROWN[3]; [1]Neurosci., Lake Forest Col., Lake Forest, IL; [2]The Chicago Med. Sch., [3]Sch. of Grad. and Postdoctoral Studies, Rosalind Franklin Univ., North Chicago, IL

Abstract: The aeolid nudibranch *Berghia stephanieae* is a recently introduced model species being studied as part of a collaborative, NIH BRAIN Initiative project aimed at developing the first transgenic lines of a gastropod mollusk. With a central nervous system containing fewer than 10,000 neurons, *Berghia* represents an attractive system in which the correlations between

genes, brain, and behavior can be readily interrogated and manipulated at the level of individual neurons and complete neural circuits. Here, we characterize the most prominent of *Berghia*'s defensive behaviors, "bristling," which involves the rapid, targeted, coordinated elevation of the cerata, dozens of appendages emerging from the animal's dorsum, and draw basic inferences about the behavior's neural bases. *Berghia* internalizes nematocysts digested from its anemone prey into the tips of its cerata; bristling thus deters prospective predators by directing these noxious cells towards an offending stimulus, with the behavior likewise being observed during *Berghia*'s own predatory activities. In the present study, bristling and concomitant behaviors, such as body shortening and avoidance turning, were elicited through either aversive tactile or chemical stimuli (a poke from a von Frye hair or 1 M NaCl, respectively) applied to different parts of the body. Kinematic analysis of tactile stimulation trials in immobilized but freely bristling animals revealed that 1) the bristling response, although always initiated close to the stimulus, then propagated from the head to the tail, even when more caudal body parts were stimulated; 2) stimulation of decerebrated specimens evoked local bristling but abolished coordinated, full-body responses; and 3) repeated stimulation of individual body loci induced response habituation. Together, these observations implicate the peripheral and central nervous systems in mediating early and late components of the bristling response, respectively. Chemical stimulation of freely locomoting animals similarly provoked bristling behavior with an accompanying modulation of locomotion: stimulation of the head drove body shortening, followed by an abrupt turning away from the stimulus, while tail stimulation accelerated forward crawling. The findings in this study elucidate a repertoire of stereotyped behaviors whose neural correlates can be further characterized in upcoming neurophysiological investigations involving large-scale optical imaging of *Berghia*'s nervous system.

Disclosures: O.H. Berg: None. **A. Boutko:** None. **V.K. Mistry:** None. **W.N. Frost:** None. **J.W. Brown:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.06

Topic: F.01. Neuroethology

Support: WVCTSI Grant U54GM104942
NSF Grant 2015317

Title: Force feedback in walking uphill and downhill: mechanosensory encoding of 'naturalistic' joint torques in stick insects

Authors: *S. ZILL[1], C. J. DALLMANN[2], N. S. SZCZECINSKI[3];
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Washington, Seattle, WA; [3]Dept. of Mechanical and Aerospace Engin., West Virginia Univ., Morgantown, WV

Abstract: Force feedback could be valuable in adapting walking to diverse terrains but the effects of changes in substrate inclination on sensory discharges have not been examined. In insects, force feedback is provided by campaniform sensilla, mechanoreceptors that monitor forces as cuticular strains. In previous studies, we have examined how campaniform sensilla on the stick insect tibia encode 'naturalistic' force stimuli derived from joint torques measured in animals walking freely on level ground. Here, we examine their responses to torques at the hind leg femur-tibia (FT) joint measured during uphill and downhill walking (Dallmann et al., J. Exp. Biol. 222, 2019). Dallmann et al. showed that FT torques are quite variable during level walking (including changes in torque direction from flexion to extension), but that torques are larger in magnitude and more consistent when traversing sloped surfaces: animals use predominately extension torque in propulsion on uphill slopes and consistently flexion torques to brake forward motion when going downhill. In the present study, we analyze the FT torques in more detail and record the responses of campaniform sensilla to the mean torques and the torques of individual steps that showed large variations (as naturally occurring 'perturbations'). We find that in level walking, FT torques in different directions tend to occur in different ranges of joint angles (flexion torques in ranges of joint extension, extension torques in ranges of joint flexion). In slope walking, FT torques reflect both the gravitational vector (Fz) and braking/accelerating forces exerted in the walking direction (Fx). The FT joint also generates large lateral forces (Fy) in downhill walking that could enhance substrate grip. Preliminary extracellular recordings of campaniform sensilla in response to these torques indicate that sensory signals encode the torque direction and strongly reflect the rate of change of force (dF/dt) in walking on all substrates. Discharges also reflect the force magnitude on slopes but are modulated and follow dF/dt. These findings are now being studied in a model that accurately simulates the properties of leg campaniform sensilla (Szczecinski et al., Living Machines, 2020). Future experiments are planned to examine the effects of sensilla on motor activities. Our working hypothesis is that sensory feedback indicating force dynamics (dF/dt) at the FT joint in the hindleg can be used to stabilize and adapt walking to diverse terrains.

Disclosures: S. Zill: None. **C.J. Dallmann:** None. **N.S. Szczecinski:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.07

Topic: F.01. Neuroethology

Support: Chellgren Endowed Funding to R. L. C.

Title: Behavioral effects to heat in the blow fly (Phaenicia sericata)

Authors: *N. MARGUERITE[1], R. L. COOPER[1], D. HARRISON[1], D. HARRIS[2];
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Abstract: Larvae of Drosophila and other related insects, such as the medicinal blow fly (*Phaenicia sericata*), demonstrate a thermal preference which varies among species. What drives the differences in behavioral response among species is not fully understood but is likely driven by evolutional adaptive pressure. The larvae of *Phaenicia sericata* when placed in the cold, after a warm exposure, will aggregate which is assumed to be for warmth. Additionally, the larvae of *Phaenicia sericata* are attracted to heat when placed in a cold environment (10^{o}C). Neither aggregation or heat attraction are robust in larval *Drosophila melanogaster* with or without over expression of the TRPA1 receptor (i.e. the capsaicin or heat receptor) in sensory neurons. Larval *Drosophila* with over expression of genes coding for TRPA1 in sensory neurons are repelled by capsaicin or high heat (>28^{o}C), whereas the non-over expressers show no behavioral response to capsaicin or heat. The larvae of *Phaenicia sericata* are attracted to heat over a food source at 20^{o}C but showed no preference at 24-28^{o}C. Larvae Drosophila, with over expression of the TRPA1 receptor, reveal robust avoidance to heat or capsaicin even over starvation. These differences in behavior, thought to be driven by TRPA1 expression, may be a survival skill for *Phaenicia sericata* to find decaying animal material or a cooperative group when exposed to cold.

Disclosures: N. Marguerite: None. **R.L. Cooper:** None. **D. Harrison:** None. **D. Harris:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.08

Topic: F.01. Neuroethology

Support: NIH Grant R35GM124883
NSF Graduate Research Fellowship Program Grant to to J.M.

Title: Towards a computational ethology of web-making: quantifying movement sequences underlying stages of spider web-building

Authors: *A. CORVER[1], N. WILKERSON[3], J. D. MILLER[2], A. GORDUS[2];
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Abstract: The geometric complexity and stereotypy of spider webs have long generated interest in their algorithmic origin. Like other examples of animal architecture, web construction is the

result of several assembly phases that reflect different behavioral stages that must be properly coordinated to build a successful structure. Manual observations have revealed a range of sensory cues and movement patterns used during web construction, but methods to systematically quantify the dynamics of these sensorimotor patterns are lacking. Here, we apply an analytical pipeline to the web-making behavior of the orb weaver Uloborus diversus. Using an unsupervised clustering approach, we identify stereotyped leg movements both specific to and shared across stages of construction. A Hierarchical Hidden Markov Model reveals that stages of web-building are characterized by distinct and stereotyped sequences of actions largely shared across individuals. We will also share progress on ongoing work using semi-automated annotation of dynamic web geometry to model the spider's tactile sensory experience during web-making, and our progress towards developing two-photon calcium imaging in this spider.

Disclosures: A. Corver: None. **N. Wilkerson:** None. **J.D. Miller:** None. **A. Gordus:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.09

Topic: F.01. Neuroethology

Support: NSF grant HRD 1137725

Title: Acute and chronic anthropogenic noise exposure potentially affect Coenobita clypeatus' investigation and exploration of shell resources

Authors: *P. N. NEGRON-MORENO[1,2], A. D. GONZÁLEZ-COLÓN[2], F. J. TORRES-TORRES[2], N. RIVERA-ROSADO[2], A. FIGUEROA-GONZÁLEZ[2], N. RODRIGUEZ-MERCADO[2], M. A. GONZÁLEZ- DE JESÚS[2], L. L. VILLALOBOS-MILLAN[2], M. ROSADO-BURGOS[2], M. I. DE JESÚS-BURGOS[2];
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Abstract: Noise pollution is recognized as a significant global environmental stressor. Studies suggest that noise exposure can alter animal behaviors related to reproduction, mating, resource assessment, and decision-making. The natural soundscape of littoral ecosystems is mainly affected by anthropogenic noise derived from tourism, urban development, and related activities. *Coenobita clypeatus* is a species abundant in the littoral zones of tropical and subtropical regions, that relies on basic behavioral patterns for survival. In particular, shell selection requires crabs to analyze the size, shape, nutrient content, and weight of the shell to decide whether it is worth acquiring as a resource. While the detrimental effects of noise pollution on animal behaviors are known, the implications of noise pollution on *C. clypeatus'* ability to gather information and assess resources remains unclear. We hypothesized

that exposure to anthropogenic noise would impair *C. clypeatus'* investigative patterns and decision-making abilities during shell selection. To evaluate the effects of noise exposure on *C. clypeatus'* shell selection behavior, animals were subjected to either an acute or chronic noise regimen. We analyzed the free exploration and investigation of a suboptimal, an optimal, and a supraoptimal shell resource by control, chronically- and acute-noise exposed animals. Preliminary results suggest that most animals regardless of the noise conditions opted for the optimal shell. Interestingly, animals that opted for suboptimal shells were only animals under chronic and acute noise regimens. These animals also spent more time investigating and occupying the suboptimal shell compared to animals under control conditions ($p<0.05$). Overall, these data seem to suggest that animals under noise exposure, either acute or chronic noise, display changes in their processing of investigation and exploration of key resources for survival. Future studies are necessary to provide a better understanding of the potentially detrimental effects of anthropogenic noise on the behavior and the neural substrates underpinning littoral organisms' behavior.

Disclosures: P.N. Negron-Moreno: None. **A.D. González-Colón:** None. **F.J. Torres-Torres:** None. **N. Rivera-Rosado:** None. **A. Figueroa-González:** None. **N. Rodriguez-Mercado:** None. **M.A. González- De Jesús:** None. **L.L. Villalobos-Millan:** None. **M. Rosado-Burgos:** None. **M.I. De Jesús-Burgos:** None.

Poster

P576. Behavioral Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P576.10

Topic: F.01. Neuroethology

Support: University of the South Conduff Fund
Sewanee Undergraduate Research Fellowship

Title: Can't right, won't right - the differing roles of neurotransmitters in sea urchin righting behavior

Authors: M. MCDONALD, N. P. GRIFFIN, E. HOWELL, D. LI, S. HARNEW-SPRADLEY, P. RODRIGUEZ, A. LANCASTER, F. UMUTONI, J. BESH, ***C. SHELLEY**;
Univ. of the South, Sewanee, TN

Abstract: Sea urchins and other echinoderms occupy an unusual position in animal neurobiology as they lack a brain or a brain-like structure, but phylogenetically they are more closely related to vertebrates than virtually all other invertebrates. Despite the use of sea urchin gametes and larvae in developmental biology for over one hundred years, and the simplicity of the adult (metamorphosed) sea urchin nervous system, very little is known about how the adult

sea urchin nervous system functions, and in particular is utilized to produce behavior. One such behavior is the righting response, in which an inverted sea urchin rights itself within a few minutes. Here we determine roles in the righting response of some of the major neurotransmitters using antagonists of nicotinic acetylcholine, $GABA_A$, glycine, dopamine, and norepinephrine receptors. Antagonists of these receptors slowed the righting response in dose-dependent manners, with IC50s for righting, in order of potency of, 4.3 μM for glycine receptors, 10.7 μM for dopamine receptors, 39.8 μM for adrenergic receptors, 173 μM for $GABA_A$ receptors, and 7.8 mM for nicotinic acetylcholine receptors. It is also shown that inhibition of both glycine and adrenergic receptors inhibits tube foot movement independent of righting, and it may be that this inhibition of tube foot movement is responsible for the slowed righting seen when these receptors are inhibited. Conversely, although inhibition of dopamine receptors slowed the righting response, this inhibition had no effect on tube foot motility, indicating that these receptors play larger roles in the neural processing involved in the righting behavior, rather than the actual physical righting. Given dopamine's well characterized roles in motivation, these results raise the question of whether inhibiting dopaminergic signaling in sea urchins renders them unable to right (at the level of neural processing), or removes the motivation to right, and further raises the question of whether in this context, at the level of neural processing, there is actually any difference between these two states.

Disclosures: M. McDonald: None. **N.P. Griffin:** None. **E. Howell:** None. **D. Li:** None. **S. Harnew-Spradley:** None. **P. Rodriguez:** None. **A. Lancaster:** None. **F. Umutoni:** None. **J. Besh:** None. **C. Shelley:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.01

Topic: H.03. Decision Making

Title: Two-photon calcium imaging of orbitofrontal cortex in mice performing economic choices reveals stable activity profiles over many weeks

Authors: *A. LIVI, M. ZHANG, T. E. HOLY, C. PADOA-SCHIOPPA;
Washington Univ. of St Louis, Saint Louis, MO

Abstract: Lesion and neurophysiology studies established a strong link between economic choice and the orbitofrontal cortex (OFC). In particular, studies where monkeys or mice chose between different juices identified in OFC different groups of neurons encoding individual offer values, the binary choice outcome and the chosen value (Padoa-Schioppa and Assad, 2006; Kuwabara et al., 2020). It was proposed that these cell groups form a decision circuit, but the organization and mechanisms of this circuit are poorly understood. To shed light on this

fundamental question, we developed a new approach for two-photon calcium (Ca2+) imaging of OFC while mice engage in economic choices.In a single surgery, we inject AAV to express GCaMP6f and we implant a GRIN lens (diameter: 1 mm; A2.6 mm and L1.5 mm from Bregma). In each animal, we can thus record from 4-6 independent fields of view (FOVs). In each FOV, we routinely isolate 60-140 cells. We process Ca2+ activity with the CaImAn package (Pnevmatikakis et al., 2016; Giovannucci et al., 2019). For each cell, fluorescence traces are normalized by calculating $\Delta F/F$. Crucially, we can record from the same FOV in multiple sessions (different days). To match neurons, we register sessions to a common template, use locations defined in one session as reference for other sessions, and impose that $\geq$70% of cells be identified in all sessions. Using this approach, we can examine whether and how the neural population in OFC reorganizes over time.During the experiments, head-fixed mice choose between two juices (labeled A and B) offered in variable amounts. Each juice is associated with an odor, and the odor concentration indicates the juice quantity. In each trial, the two odors are presented simultaneously from the left and right sides, and the animal indicates its choice by licking a juice spout. The association between the juice type (A, B) and the spatial direction (left, right) varies pseudo-randomly. In each session, behavioral data are analyzed with a logit regression, which provides an estimate for the relative value of the juices. We recorded from 4 mice (21 FOVs; >1000 cells). Recordings started the first day in the choice task and continued for $\leq$4 months. We examined the activity profile of individual cells over the course of the trial. A cosine product quantified the similarity between activity profiles. We thus examined the distribution of cosine similarities obtained for repeated measures of the same cell and compared it with that obtained for different cells. The difference between the two distributions was highly significant even when sessions occurred >8 weeks apart. Hence, the activity profiles of individual neurons remained stable over time.

Disclosures: A. Livi: None. **M. Zhang:** None. **T.E. Holy:** None. **C. Padoa-Schioppa:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.02

Topic: H.03. Decision Making

Support: McDonnell Center for Systems Neuroscience pre-doctoral fellowship

Title: Neuronal encoding of economic decision variables in the mouse orbitofrontal cortex is consistent across days

Authors: *M. ZHANG, A. LIVI, T. E. HOLY, C. PADOA-SCHIOPPA;
Neurosci. Dept., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: An extensive literature indicates that the orbitofrontal cortex (OFC) plays an important role in economic choice behavior. In particular, neurophysiology studies where monkeys or mice chose between different juices identified in OFC different groups of cells encoding the value of individual options, the binary choice outcome, and the chosen value. These variables capture the input and output of the choice process, suggesting that OFC may host a decision circuit. To shed light on the organization of this circuit, we developed a new approach for two-photon calcium imaging of OFC in mice choosing between different juices (see companion abstract by Livi, Zhang et al.). Here we investigated whether the functional properties of individual neurons are stable over time.

We recorded from 3 mice and 10 distinct field of views (FOVs). Each FOV was imaged multiple times and we identified the same cell across sessions. Our data set included 767 neurons, each recorded twice and >7 days apart. Following the procedures of previous studies (Kuwabara et al., 2020), each cell was analyzed in relation to several decision variables. Tuned cells were classified independently in each session as encoding the spatial configuration, the ipsilateral offer value, the chosen side, or the chosen value. For a population analysis, we constructed a contingency table in which rows and columns corresponded to classes defined in the two sessions, and entries represented cell counts. We also derived a table of odds ratios. For all entries on the main diagonal, odds ratios were significantly above chance. Conversely, the vast majority of entries off the main diagonal were at or below chance level ($p<0.01$; Fisher's exact test). In other words, classification in the two sessions was generally very consistent, indicating that cells' functional properties are preserved over time.

Next, we examined whether neurons with similar functional properties form anatomical clusters. For each FOV, we calculated the spatial distance between each pair of cells encoding a particular variable. The resulting distribution was statistically indistinguishable from that obtained pairing cells in the same FOV randomly ($p>0.05$, one-way ANOVA test). Similarly, the distributions obtained for different cell groups were statistically indistinguishable. In other words, we did not find any evidence for spatial clustering.

In summary, our results indicate that the functional properties of OFC neurons encoding different decision variables are stable in time and not anatomically segregated. These findings and our approach pave the way to investigate in greater depth the neural circuit underlying economic decisions.

Disclosures: M. Zhang: None. **A. Livi:** None. **T.E. Holy:** None. **C. Padoa-Schioppa:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.03

Topic: H.03. Decision Making

Support: NSF GRFP 20192862620 (Aguirre)
NIH K01 DA042219 (P.R.O.)
NIH R01DA047870 (Soltani/Izquierdo)
5F31AA028183-02 (Munier)

Title: Dissociable roles of basolateral amygdala and ventrolateral orbitofrontal cortex in flexible stimulus outcome and action-outcome association learning

Authors: *C. G. AGUIRRE[1], M. GOLDFARB[1], K. DAS[1], T. YE[1], J. J. MUNIER[2], M. GOMEZ[1], P. R. O'NEILL[3], I. SPIGELMAN[2], A. IZQUIERDO[1];
[1]Psychology, [2]Div. of Oral Biol. & Medicine, Sch. of Dent., UCLA, Los Angeles, CA; [3]Shirley and Stefan Hatos Ctr. for Neuropharmacology, Semel Inst., Dept. of Psychiatry and Biobehavioral Sciences, Univ. of California, Los Angeles, Los Angeles, CA

Abstract: Reversal learning methods in rodents often require animals to form associations between the spatial side (left vs. right) and reward (action-outcome, A-O). Other task types require animals to form associations between stimuli and reward (stimulus-outcome, S-O). Here we examined the roles of ventrolateral OFC (vlOFC) and basolateral amygdala (BLA) in flexible learning of S-O and A-O associations using chemogenetic manipulation. Male and female Long-Evans rats were required to discriminate the correct stimulus/action by nose-poking a touchscreen and assessed on measures of accuracy and speed. Rats were stereotaxically injected with either bilateral inhibitory hM4Di DREADDs on a CaMKIIa promoter in vlOFC or BLA, or with an eGFP virus in these same regions. Separately, Ca^{2+} imaging in slices co-expressing GCaMP6f and hM4Di DREADDs in BLA confirmed reduced activity following application of clozapine-N-oxide (CNO). vlOFC slice validation is ongoing. Rats were administered either CNO or vehicle (VEH) solution (3mg/kg, i.p.) 30 min prior to testing during reversal learning, using a within-subject, counterbalanced design for drug administration. Rats learned the initial S-O more slowly than A-O associations (S-O, 10.64 1.18; A-O, 2.250.11 sessions to 75%). During reversal phases, rats required more sessions to reach criterion on the S-O task relative to the A-O task, especially when vlOFC was inactivated, and exhibited poorer accuracy in S-O compared to A-O learning, with effects in the same direction (vlOFC poorer than BLA group). Rats were slower to initiate trials and to choose the correct and incorrect options in S-O than A-O learning (BLA slower than vlOFC group). Rats also took longer to collect reward after making a correct choice in A-O compared to S-O learning (BLA slower than vlOFC group). Overall, BLA inhibition enhanced A-O learning, whereas vlOFC inhibition slowed A-O learning. Finally, we fitted S-O and A-O reversal choice behavior with reinforcement learning models and found that a model with a single learning rate parameter (model 1) better captured S-O than A-O learning, and better than a model with two learning rates (model 2). There was a higher learning rate in S-O than in A-O across most virus/drug conditions. Animals exhibited greater Win-Stay strategy in A-O compared to S-O, consistent with a greater inverse temperature parameter in the A-O task in both model 1 and model 2. Overall, the pattern of results suggests different mechanisms for involvement of vlOFC and BLA in flexible learning, dependent on the nature of the associations, and reveal a need for different, more sensitive metrics to capture region-specific contributions.

Disclosures: C.G. Aguirre: None. **M. Goldfarb:** None. **K. Das:** None. **T. Ye:** None. **J.J. Munier:** None. **M. Gomez:** None. **P.R. O'neill:** None. **I. Spigelman:** None. **A. Izquierdo:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.04

Topic: H.03. Decision Making

Support: NIH Grant R01DA047870-03S1
NIH Grant R21MH122800

Title: Contributions of ventrolateral orbitofrontal cortex to flexible stimulus-reward learning under delay conditions

Authors: *J. ROMERO SOSA[1], E. TREPKA[2], A. SOLTANI[2], A. IZQUIERDO[1];
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Abstract: Stimulus-outcome learning can be influenced by changes in different reward attributes such as probability-of-reward or delay-to-reward, but the relationship between the two is unclear. To address this, we probed if stimulus-delay associations are learned similarly to stimulus-reward associations and the role and the role of the ventrolateral orbitofrontal cortex (vlOFC) in such learning. To that end, we trained male and female Long-Evans rats to discriminate two equiluminant visual stimuli, S_A and S_B, displayed on a touchscreen in pseudorandom left/right positions under two conditions: stimulus-reward discrimination where S_A results in reward (p=1.00) and S_B does not (p=0.00), both with a 1 s reward delay; or stimulus-delay discrimination where S_A results in a shorter wait time (0s) than S_B (8 s), both with 100% reward probability. Rats were prepared with bilateral inhibitory (hM4Di, G_i) DREADDs or eGFP null virus on a CaMKIIa promoter in vlOFC, and administered either clozapine-N-oxide (CNO) or vehicle (VEH) solution (3mg/kg, i.p.) 30 min prior to testing on all sessions following the initial discrimination phase. Rats learned stimulus-delay faster than stimulus-reward discrimination even when reward probabilities were fully predictive. To equate reward rate between stimulus-reward and stimulus-delay tasks, we introduced catch trials in the latter wherein 45% of all trials, regardless of which stimulus was chosen, were unrewarded. Introducing this uncertainty decreased stimulus-delay discrimination performance, flattening the learning curve. We then conducted a mixed-effects GLM with drug, virus, block, session, and task type (stimulus-reward vs. stimulus-delay) as predictors of accuracy. Our preliminary data suggest that vlOFC inhibition (hM4Di-CNO) enhances the choice of the stimulus associated with a shorter wait time. Finally, to probe behavioral strategy, we performed additional analyses using new entropy-based metrics

(Trepka et al. biorxiv) on each learning block's first and last sessions. Specifically, we compared surprise in animals' response based on association of outcome with chosen stimulus or chosen side, and separately after rewarded and unrewarded trials. We found that measures of entropy based on stimulus decreased on the last relative to the first session of the catch trial blocks, indicating that accumulation of experience on the contingencies leads to a more consistent response to reward. On the other hand, entropy based on side remained high throughout learning. Ongoing analyses are aimed at comparing hM4Di and eGFP groups on these measures across both probability and delay tasks.

Disclosures: J. Romero Sosa: None. **E. Trepka:** None. **A. Soltani:** None. **A. Izquierdo:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.05

Topic: H.03. Decision Making

Title: It takes a model to beat a model: keeping score for the neuroeconomics-based price heuristic hypothesis vs. price theory and the efficient markets hypothesis

Authors: *J. L. HARACZ;
Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Objective: According to price theory (Jaffe et al., 2019) and the efficient markets hypothesis (EMH; Fama, 2014), supply and demand determine prices in markets that are at, or efficiently approaching, a self-stabilizing equilibrium (i.e., "price-equals-value markets" [Statman, 2017, p. 289]). This notion of price is challenged by the heuristics-biases literature, neuroeconomics, and bubble-crash instability, which suggest that prices deviate from economically fundamental values. Therefore, the present study seeks a more descriptively accurate reconceptualization of price. Methods: A systematic literature review of price theory, the EMH, neuroeconomics, heuristics, and biases used related search terms (e.g., demand curve, elasticity of demand, rule of thumb, trading, limits of arbitrage, and deliberation- or valuation-related brain areas) in the EconLit and PubMed databases, along with browsing abstracts of decision neuroscience meetings. Results: Over 30 years before Tversky and Kahneman (1971, 1974) analyzed heuristics and biases, Hall and Hitch's (1939, p. 18) survey revealed that price setters used a "full cost" "rule of thumb" rather than equating "marginal revenue and marginal cost" as in classical economic theory. This and many other examples of price-based heuristics (i.e., price heuristics) accrued before and after (e.g., price setters' "herding" [Kienzler, 2018, p. 86]) neoclassical economics came into prominence with an emphasis on price as a reliable indicator of fundamental value. In contrast, the novel price heuristic hypothesis (PHH) proposes that prices only occasionally indicate intrinsic values. Prices hypothetically act as heuristic

signals because: a) heuristics are embedded in prices (Hall & Hitch, 1939; Kienzler, 2018); b) prices guide market participants' decision making by serving as a signal for the use of heuristics. In the bubble-crash context, traders avoiding effortful deliberation (e.g., as in neural autopilot theory [Camerer et al., 2021]) could simply use asset-price changes as guides, or heuristics, to popular or unpopular assets, thereby letting traders fall under the spell of a bubble or crash bias, respectively. These biases may recruit habit-related basal ganglia mechanisms (Camerer et al., 2021), thereby promoting bubbles and crashes based on trend-following investment strategies. Conclusions: Price theory and EMH-inspired asset-pricing models are useful tools for analysts and policymakers, but they are not always descriptively accurate. The more descriptively accurate PHH is shown here to be relatable to neural mechanisms, but it has yet to be formalized as a tool for analysis and policymaking.

Disclosures: J.L. Haracz: None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.06

Topic: H.03. Decision Making

Support: UCLA Division of Life Sciences Retention Fund (Izquierdo)

Title: Dissociable roles for basolateral amygdala (BLA) and anterior cingulate cortex (ACC) in the bias for information

Authors: *V. V. GONZALEZ, M. G. GOMEZ, A. P. BLAISDELL, A. IZQUIERDO; Psychology, UCLA, Los Angeles, CA

Abstract: When given a choice between a lean alternative that contains information about the outcome of a trial versus a richer alternative without information, most animals prefer the former: they prefer information even though that alternative yields lesser reward. This effect has been referred to as the suboptimal choice phenomenon or the 'bias for information' effect and it has been observed in pigeons, starlings, rats, and in humans. Most studies have focused on better understanding the behavioral limits of this phenomenon, yet there are few experiments directed at identifying the neural substrates of this preference. Value is computed by comparing the subjective value of the alternatives, after integrating all the features of the options, and perhaps also including information value, a process that may rely on corticoamygdalar circuits. In the present set of experiments, female Long-Evans rats were prepared with either bilateral inhibitory hM4Di DREADDs on a CaMKIIa promoter in basolateral amygdala (BLA) or anterior cingulate cortex (ACC), or administered an eGFP virus in these regions. Rats were administered either clozapine-N-oxide (CNO) or vehicle (VEH) solution (3mg/kg, i.p.) 10 min prior to behavioral

testing. We used a within-subject counterbalanced design for drug administration. We first evaluated the role of BLA and ACC during acquisition and performance of the basic effect: choosing between two alternatives by pressing the right or left lever, in which choice of one (suboptimal/informative alternative) resulted in 20% of the trials followed by a tone (S+) that lasted 60s and always ended with a sucrose pellet, and on the other 80% of the trials followed by another tone (S-) lasting 60s but never resulting in reinforcement. Choice of the other alternative always resulted in a third 60-s tone (S3) followed by reward on 50% of the trials. Then, we trained a new set of tones to be S+, S- and S3 to evaluate the role of ACC and BLA in the acquisition and performance of the informative and non-informative cues in preference. Finally, we reversed the spatial location of the two levers and assessed the contribution of both areas during learning and steady-state performance. We found that inhibition of ACC, but not BLA, reversed the preference of the rats. BLA inhibition, on the other hand, produced an attenuated acquisition of the preference for information when new cues were introduced, but we found no evidence for a contribution of BLA in learning the reversed location of the two levers. In replication cohorts, we expect ACC to similarly contribute to the learning and steady-state preference of new informative and non-informative cues, and on the spatial reversal of that choice.

Disclosures: V.V. Gonzalez: None. **M.G. Gomez:** None. **A.P. Blaisdell:** None. **A. Izquierdo:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.07

Topic: H.03. Decision Making

Support: NIH R21 MH122800
UCLA Division of Life Science Retention fund

Title: Probing hippocampal involvement in stimulus- and action-based reward learning using a combination of electrophysiological, chemogenetic, and behavioral tracking approaches

Authors: *A. G. HOWE, C. G. AGUIRRE, T. YE, A. M. WIKENHEISER, A. IZQUIERDO; UCLA, Los Angeles, CA

Abstract: The hippocampus (HPC) is famous for the “place” cell; a neuron that spikes vigorously when a rat moves through a particular subregion of a given physical space. Populations of place cells are theorized to form “cognitive maps” of physical behavior space. More recent data demonstrates that HPC function extends beyond maps of physical space, demonstrating HPC representations of non-spatial behavior (Aronov et al 2017) and elements of

the task structure itself (Samborska et al. biorxiv 2021). Results from the macaque demonstrate a HPC map of non-physical "value space" (Knudsen & Wallis, biorxiv 2021). Complementing single cell observations, disruption of the Theta local field potential (LFP) in either HPC or orbitofrontal cortex (OFC) in the macaque impairs flexible reward learning (Knudsen & Wallis, 2020). However, the mechanism by which HPC is involved in flexible stimulus-based and action-based reward learning and the degree to which OFC-HPC interaction supports similar behavior in the rat is unknown. Here, we attempt functional dissection of this circuit with simultaneous behavior, electrophysiology and chemogenetic inactivation in the rat. Male and female Long-Evans rats (N=6) received bilateral inhibitory hM4Di DREADDs on a CaMKIIa promoter in ventrolateral OFC (n=4) or in HPC (n=2) at the same time as implantation of a novel 8 tetrode (32 channel) adjustable-position drive in HPC. This drive provides single-unit and LFP data in a device suitable for free-movement in an operant chamber where we could precisely mark trial events including trial initiation, response selection, and reward collection. Rats discriminated the correct stimulus or action (left/right) by nosepoking a touchscreen. Rats received either clozapine-N-oxide (CNO; 3mg/kg, i.p.) or vehicle (VEH) solution 30 min prior to testing during reversal learning, using a within-subject, counterbalanced design for drug administration. Computer vision and machine learning techniques enabled the extraction of the rat's position and behavior state from live video; TTL pulses from the behavioral system facilitated synchronization of electrophysiological data with the other data streams. We found single units in HPC which encoded specific events such as trial initiation and correct/incorrect choice in the task sequence without encoding for a specific spatial location. We also confirmed placement of electrodes in CA1, as well as DREADDs expression in OFC. Analyses of resulting DREADDs inactivation of either vlOFC or HPC on single unit activity are ongoing.

Disclosures: A.G. Howe: None. **C.G. Aguirre:** None. **T. Ye:** None. **A.M. Wikenheiser:** None. **A. Izquierdo:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.08

Topic: H.03. Decision Making

Support: NIH Grant R21MH122800
NIH Grant R01DA047870

Title: Theta oscillations in anterior cingulate and orbitofrontal cortex differentially modulate accuracy and speed in flexible stimulus- and action-based learning

Authors: *T. YE, A. M. WIKENHEISER, A. IZQUIERDO;
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Abstract: Flexible reward learning relies on frontal cortex, with substantial evidence indicating the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) play important roles. A systematic investigation of how rat ACC and OFC contribute to stimulus-based and action-based learning is lacking in the broader literature. Further, we recently suggested that a thorough consideration of both accuracy and speed during discrimination and reversal phases can be particularly informative when studying neural signatures of flexible learning (Aguirre et al., 2020; Harris et al., 2020), which we set out to study here. Recent studies in both rat and macaque suggest theta oscillations may be a spectral signature that coordinates this learning. However, causal network-level interactions between ACC and OFC in flexible learning remain unclear. We investigated the learning of stimulus-outcome (S-O) and action-outcome (A-O) associations using a combination of simultaneous *in-vivo* electrophysiology in dorsal ACC (dorsal area 32/24, van Heukelum et al. (2020)) and ventral OFC, partnered with bilateral inhibitory DREADDs in ACC. Using a within-subject design, we examined accuracy and speed across distinct and precisely-defined trial epochs including trial initiation, choice of the correct response, choice of the incorrect response, and reward collection. Using a series of mixed-effects GLMs, we found consistent and significant modulation of accuracy, not speed, by theta (5-10 Hz) power in ACC and OFC during S-O discrimination and reversal learning, respectively. Specifically, ACC and OFC theta in the discrimination post-choice epoch were significant moderators of the choice of correct stimulus. ACC theta in the pre-choice epoch was the only significant predictor of the correct choice ($p=0.01$). In reversal, OFC theta in the post-choice epoch was the only significant moderator of the choice of correct and incorrect stimulus. Correlation analyses did not reveal any significant relationships between theta power and movement speed for any of the trial epochs (all $p>0.05$). In contrast, in A-O learning an interaction of ACC x OFC theta predicted both speed and accuracy of the correct response, specifically in the reversal phase. Importantly, ACC inhibition affected A-O latency measures more profoundly than accuracy, suggesting that action-based reversal learning impairments reported in the literature may be primarily due to changes in deliberation speed. The pattern of results suggests there is less specialization of OFC and ACC (i.e., more shared roles) in flexible learning of both stimuli and actions in rats than in human/nonhuman primates.

Disclosures: T. Ye: None. **A.M. Wikenheiser:** None. **A. Izquierdo:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.09

Topic: H.05. Working Memory

Support: NIH Grant R01 5270633
COBRE 5-27132

Title: Representation of credit-deserving feature information and reward prediction errors across prefrontal cortical areas during credit assignment

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Abstract: The outcome of a choice must be attributed to a causal event or experience and properly adapted to the success or failure of that choice even the causal event is separated in time or intermingled with distractors. This is known as a credit assignment problem and the prefrontal cortex (PFC) is likely to play a major role in its solution. However, the precise areas involved, and the manner through which outcomes and credit-deserving features are integrated, are still unknown. To investigate this, we trained two monkeys to perform a credit assignment task and implanted large recording arrays (96 and 128 electrodes) over the PFC, including dorsomedial PFC (dlPFC), ventral lateral PFC (vlPFC) and Orbitofrontal Cortex (OFC). In the task, they learned to attribute a choice outcome to one of several earlier options, and correct choices were indicated by generic feedback (not revealing the causal feature) followed by juice reward. We fit a reinforcement learning model to behavior and then examined how neuronal activity weighted relevant task parameters using a multi regression analysis (parameters included cue identities, cue locations, cue values, reward prediction errors and spike history effects). Preliminary analyses revealed that vlPFC and OFC both carried information about the credit-deserving cue (14.6% and 12.4% fraction of recorded vlPFC units and 12.9% and 16.9% fraction of recorded OFC units, $p<0.05$) and about reward prediction errors (20.5% and 20.9% fraction of recorded vlPFC units and 23.6% and 19.1% fraction of recorded OFC units, $p<0.05$) during feedback period in each animal, respectively. These results indicate that both vlPFC and OFC were involved in resolving temporal credit assignment and further work will characterize interactions between credit-deserving features and reward prediction errors in these PFC areas.

Disclosures: E. Lee: None. **W.F. Asaad:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.10

Topic: H.03. Decision Making

Support: SimonsCollaboration on the Global Brain
Swartz Foundation
NIH Grant R01MH062349

Title: Multi-regional neural circuit mechanisms underlying the maintenance of action value and its reward-dependent update

Authors: *U. PEREIRA OBILINOVIC[1], H. HOU[2], K. SVOBODA[3], X.-J. WANG[1];
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Abstract: During dynamic foraging, action values are stored in short-term memory over times of several behavioral trials (seconds to minutes), much longer than intrinsic neural time scales. The stored action values bias actions to maximize reward. In the frontal cortex (mPFC, OFC), neuronal representations of action value can persist for tens of seconds. After a choice is made, mPFC firing rates are proportional to the new estimated action value (Bari et al, 2019). Two neuronal mechanisms have been proposed for the memory trace of action values and their update: (i) reward-dependent synaptic plasticity and (ii) integration of action value by network dynamics. In the first mechanism, rewards trigger dopamine release, which enables Hebbian plasticity at corticostriatal or mPFC synapses (Soltani and Wang, 2006). This plasticity rule leads to matching behavior in the steady-state, as observed in multiple species. Since spiking activity is driven by synaptic inputs, neuronal activity is modulated by action values. In the second mechanism, neural populations integrate reward events for each of the two action values: a pulsatile input proportional to the reward prediction error (RPE) in the action value mode direction changes their firing rates by a specific amount, and the updated action value is maintained in short-term memory. Similar to a theoretical proposal of mPFC as a meta-reinforcement learning system (Wang and Kurth-Nelson et al, 2018), in this second model, action values are integrated into a line-attractor without the need for synaptic plasticity. We analyze these two models drawing contrasting predictions. We test some of these predictions in electrophysiological recordings during dynamic foraging in mice.

Disclosures: U. Pereira Obilinovic: None. **H. Hou:** None. **K. Svoboda:** None. **X. Wang:** None.

Poster

P793. Orbitofrontal Cortex: Neural Circuitry of Choice Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P793.11

Topic: H.08. Learning and Memory

Support: NIDA/NIH

Title: The orbitofrontal cortex is necessary for forming outcome-specific representations during learning

Authors: *K. M. COSTA[1], R. SCHOLZ[2], K. LLOYD[2], M. P. GARDNER[3], P. DAYAN[2], G. SCHOENBAUM[1];
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Abstract: The orbitofrontal cortex (OFC) is necessary for using learned representations of outcomes to guide behavior, i.e. model-based cognition. For example, lesions of the OFC have long been known to impair changes in conditioned responding following reinforcer devaluation. These findings, among others, have fueled the hypothesis that the OFC contributes to the use of mental representations that link cues to the incentive properties of outcomes and support inferential thinking (which is necessary for reinforcer devaluation effects). However, it is unclear whether the OFC is necessary for creating such representations or only for their use in guiding subsequent decision making. To answer this question, we conducted a simple experiment in which we used high-potency chemogenetics to inactivate OFC in rats only during the first, rather than in the final, phase of an outcome-specific Pavlovian reinforcer devaluation task. Rats were conditioned to associate two cues with two different rewards, during which OFC was inactivated in the experimental group. Subsequently, one reward was devalued by pairing with LiCl, and, finally, responding to the two cues was assessed in an extinction test. During conditioning, groups showed robust learning to both cues, although the experimental group responded less in the final two sessions. Both groups also similarly reduced consumption of the outcome paired with illness in taste aversion training. However, in the probe test, rats in the experimental group responded equally to cues predicting the devalued versus the non-devalued outcome. Importantly, they suppressed responding to both cues, indicating a loss in response specificity rather than an abolishment of devaluation effects. One interpretation is that, without OFC, rats were still able to form a model, but one without outcome-specific information. Therefore, transient and reversible OFC inactivation during conditioning affected the rats' ability to use the learned information to support selective response changes in the probe test. This result implicates OFC in the formation of appropriate model-based and outcome-specific representations during cue-outcome learning.

Disclosures: K.M. Costa: None. **R. Scholz:** None. **K. Lloyd:** None. **M.P. Gardner:** None. **P. Dayan:** None. **G. Schoenbaum:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.01

Topic: H.07. Long-Term Memory

Support: NIH Grant T32NS047987
NIH Grant 2T32MH067564
NSF Grant BCS-1921678
NSF Grant BCS-1829414

Title: Memory reactivation with sleep disruption harms specific memories

Authors: *N. W. WHITMORE, K. A. PALLER;
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Abstract: Sleep plays a role in stabilizing memories so they are not forgotten. A prevalent view is that this process depends on active consolidation during sleep; important memories are replayed in the hippocampus and cortex to drive the neural plasticity underlying consolidation. In recent years, Targeted Memory Reactivation (TMR) has emerged as a useful tool for investigating consolidation, allowing researchers to induce replay of specific memories using sensory cues presented during sleep. Memories are typically strengthened by TMR compared to control conditions. However, in some cases the opposite effect has been observed, with relatively increased forgetting for reactivated memories. We hypothesized that forgetting can occur when micro-arousals disrupt the consolidation process, perhaps due to noise injected into the memory trace at the time of reactivation. To test this hypothesis, we conducted an experiment in which 25 participants each learned 75 object-location associations before an afternoon nap. When each object appeared on the screen, its characteristic sound was also presented. Memory was tested by requiring participants to recall the screen location of each object. During the nap, 50 of the associations were reactivated during nonREM sleep using their corresponding sounds. Each sound was presented once during the nap, in random order. Half of these sounds were played at a relatively high volume, to disrupt sleep, and half were played quietly, to avoid any arousal from sleep. Participants recalled object locations both before and after the nap. The usual TMR effect, confirmed in a recent meta-analysis (Hu et al., 2019, Psychological Bulletin), is that locations are recalled more accurately following reactivation. We found a reversed TMR effect in the context of disrupted sleep, with less-accurate recall for reactivated object locations compared to those not reactivated. Sleep EEG revealed that only sounds that produced an arousal degraded memory. These findings showing that reactivation during sleep can harm specific memories have implications for understanding memory consolidation, for considering how to maximize the benefits of memory reactivation during sleep, and perhaps for constructing interventions to apply when weakening a memory would be appropriate

Disclosures: N.W. Whitmore: None. **K.A. Paller:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.02

Topic: H.07. Long-Term Memory

Support: K99 MH122663-01A1

Title: The role of encoding context in memory reactivation during sleep

Authors: *E. SCHECHTMAN, J. HEILBERG, K. A. PALLER;
Dept. of Psychology, Northwestern Univ., Evanston, IL

Abstract: Throughout our waking lives, new memories are formed continuously. Such memories never exist in a vacuum; they may be interlinked with other memories that share semantic features and with those that share temporal proximity. In this study, we explored the role encoding context plays in offline consolidation during sleep. Memory was tested using 72 images of single objects, each presented with a related sound. Human participants first formed idiosyncratic narratives linking sets of objects together. Then, they learned the distinct 2D spatial location of each object. During non-rapid-eye-movement sleep, some object-specific sounds were unobtrusively presented, a technique termed targeted memory reactivation. This manipulation putatively reactivated object memories, thereby provoking consolidation. Results showed that sleep-related changes in memory for contextually linked memories were correlated, indicating that consolidation is impacted by encoding context. Moreover, multivariate pattern analyses revealed that context-specific electrophysiological patterns were reinstated following sound presentations during sleep. Overall, these results indicate that reactivation of memories during sleep is accompanied by the reactivation of features of encoding context and that this intervening processing impacts subsequent recall.

Disclosures: E. Schechtman: None. **J. Heilberg:** None. **K.A. Paller:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.03

Topic: H.07. Long-Term Memory

Support: NIH R01 MH069456

Title: A neural network model of sleep-dependent consolidation via autonomous hippocampal-cortical replay

Authors: *D. SINGH[1], K. A. NORMAN[2], A. C. SCHAPIRO[1];
[1]Psychology, Univ. of Pennsylvania, Philadelphia, PA; [2]Psychology, Princeton Univ., Princeton, NJ

Abstract: The Complementary Learning Systems theory posits that the hippocampus "teaches" neocortex about recent experiences during offline periods, including sleep (McClelland, et al., 1995, Psychol. Rev.). How are these systems able to interact during periods with no environmental input to accomplish useful learning and shifts in representation? We present a neural network model of autonomous replay that provides an account of how the hippocampus helps neocortex build representations of recent experience during sleep, and how this new information becomes integrated into existing neocortical knowledge structures. The network architecture incorporates biological details of hippocampal circuitry and includes a neocortical layer as the target of consolidation. To simulate sleep, we silence all external input and allow the model to move autonomously among memory attractors. We leverage sleep oscillations to reveal weaknesses in the attractors (Norman et al., 2005, Neural Netw.) and allow error-driven learning. Our first simulation examined how new information stored in the hippocampus shapes neocortical representations during Slow Wave Sleep (SWS). We simulated a human category learning study that found that memory for features shared among exemplars within a category improved across a night of sleep (Schapiro et al. 2017, Sci. Rep.). In our model, pre-sleep behavior was mainly supported by the fast-learning hippocampus, and hippocampal-cortical replay during simulated SWS helped to build neocortical representations. These new neocortical representations especially benefited the shared category structure, providing an account of the human data. Our second simulation explored how sleep may help integrate new information into existing knowledge, operationalized as a continual learning problem. We adopted the classic AB-AC list learning task. We first allowed AB knowledge to be consolidated in cortex, then evaluated whether interleaving SWS and Rapid Eye Movement (REM) sleep across a night of sleep could help avoid interference from new AC learning. SWS emphasized hippocampus teaching cortex AC information while REM involved cortex replaying its previously-learned AB information, helping to protect AB representations from catastrophic interference. Alternating between SWS and REM over the course of the night may thus permit effective continual learning. Together, our simulations demonstrate key mechanisms by which autonomous replay during sleep may facilitate the construction and maintenance of neocortical knowledge.

Disclosures: D. Singh: None. **K.A. Norman:** None. **A.C. Schapiro:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.04

Topic: H.07. Long-Term Memory

Support: National Science Foundation Award 1756473
US-Israel Binational Science Foundation Award 2016244
National Institute of Neurological Disorders and Stroke UO1-NS108930
National Institute of Neurological Disorders and Stroke R01-NS084017
European Research Council ERC-2019-CoG 864353
Postdoctoral fellowship of the Human Frontier Science Program Organization
Postdoctoral fellowship of the Rothschild Foundation

Title: Hippocampal-prefrontal neuronal synchrony during human sleep mediates memory consolidation

Authors: *M. GEVA-SAGIV[1,2,4], E. A. MANKIN[2], D. ELIASHIV[3], S. EPSTEIN[5], N. CHERRY[2], G. KALENDER[2], N. TCHEMODANOV[2], Y. NIR[5], I. FRIED[2];
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Abstract: Systems-level memory consolidation during sleep is thought to depend on coordinated interplay of neocortical slow-waves, thalamo-cortical sleep spindles, and hippocampal ripples, but direct evidence is lacking. Here, we directly addressed this hypothesis by dynamically manipulating the temporal coordination between sleep activities in human medial temporal lobe (MTL) and neocortex. We implemented real-time closed-loop (RTCL) deep brain electrical stimulation in prefrontal cortex during MTL active periods, and tested how this intervention affects sleep electrophysiology and overnight consolidation of declarative memory. Upon informed consent, 14 pharmacoresistant epilepsy patients implanted with intracranial electrodes for clinical monitoring participated in intracranial stimulation and iEEG recording sessions of detailed neural activity during sleep. We examined the cognitive effects of this intervention in a subset of patients (N = 10) who learned paired associations between images in the evening and performed recognition tests immediately after learning and in the morning following sleep. Each patient participated in two night sessions (order counterbalanced) either with RTCL stimulation during sleep or without. RTCL stimulation, robustly locked to MTL slow-wave active phase, enhanced the synchronization between MTL-ripples, neocortical SW and sleep spindles (*P = 0.013). Moreover, neuronal units across multiple neocortical sites significantly synchronized their firing to MTL slow-wave activity, evident by increased phase locking to MTL field potentials (N = 64 neo-cortical units, $P<10^{-13}$). Enhancing the endogenous coordination between MTL ripples, neocortical slow waves and spindles by timed electrical stimulation resulted in higher memory accuracy on the next day (N=7 patients, $P < 0.05$). Importantly, we found a strong correlation between the extent of enhanced triple co-occurrence of MTL ripples and neocortical slow waves and spindles with overnight memory performance (N=8 patients, $\rho = 0.82$, *P = 0.017). Conversely, cognitive improvement wasn't correlated to a change in interictal spikes activity (P = 0.15). A control group (N = 3) with deep brain stimulation that was not locked to MTL active phase (but otherwise identical) did not show the electrophysiology and cognitive effects. Our results implicate fine temporal coordination between MTL ripples and neocortical slow waves and spindles as mediating mechanisms for declarative memory consolidation, and suggest potential avenues for treatment of memory disorders during sleep.

Disclosures: M. Geva-Sagiv: None. **E.A. Mankin:** None. **D. Eliashiv:** None. **S. Epstein:** None. **N. Cherry:** None. **G. Kalender:** None. **N. Tchemodanov:** None. **Y. Nir:** None. **I. Fried:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.05

Topic: H.07. Long-Term Memory

Support: R01MH111439
NSFGRFP

Title: Breaking down neural correlates of memory during sleep: An exploration of typical and atypical sleep oscillations

Authors: *E. ESPINAL[1,2], A. MISHRA[1], N. MARKOWITZ[1], E. G. CHRYSIKOU[2], A. D. MEHTA[1], S. BICKEL[1];
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Abstract: Memory is a critical piece of the human experience and impairments in neural memory networks can have devasting consequences for the affected person. A subtype of memory, episodic memory generates context for the present based on past experience and allows us to make predictions about the future. It is thought that consolidation of episodic memory requires a dynamic interplay between connected hippocampal-cortical networks, a process which crucially continues during sleep. Hallmark sleep oscillations, slow oscillations and thalamocortical spindles, couple with hippocampal sharp wave ripples (SWR) were proposed to be mechanistically involved in establishing this cortical-subcortical dialog necessary for long-term memory consolidation. The current study aimed to determine alterations in typical sleep oscillations and oscillation coupling in patients with structural hippocampal damage and correlate them with neuropsychological measures. We used intracranial electroencephalography (iEEG) in 8 patients with epilepsy to directly record hippocampal and neocortical oscillations and neuropsychological measures obtained prior to implantation. Participants in this study were diagnosed with mesial temporal sclerosis in the left hippocampus and healthy tissue in the right hippocampus. We analyzed hippocampal SWR output from both hippocampi for each participant and found significant differences in the frequency, amplitude, and waveforms of SWR in the sclerotic versus healthy hippocampi. Additionally, we found differences between hemispheres in coupling of SWRs to spindles and SOs. To understand the relationship between electrophysiological biomarkers and memory performance, we correlated outcomes with behavioral measures captured through an auditory verbal learning task designed to assess long-term memory function. Results from this work will aid in establishing a criterion for

characterizing a relationship between subcortical and cortical oscillations as they relate to memory performance. Besides aiding our understanding of the neural mechanisms underpinning memory consolidation this will ideally help with developing a neurophysiological biomarkers that may predict possible memory decline in resective or ablative neurosurgery even if no structural lesion is present.

Disclosures: E. Espinal: None. **A. Mishra:** None. **N. Markowitz:** None. **E.G. Chrysikou:** None. **A.D. Mehta:** None. **S. Bickel:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.06

Topic: H.07. Long-Term Memory

Support: Support for this research was provided by BrainsCAN at Western University through the Canada First Research Excellence Fund (CFREF)

Title: Waveform detection by deep learning reveals multi-area spindles that are selectively modulated by memory load

Authors: *M. H. MOFRAD[1,2], G. GILMORE[2,3], S. M. MIRSATTARI[4,5,6,7], J. G. BURNEO[4,8], D. A. STEVEN[4,8], A. KHAN[2,3,6,9], A. SULLER MART[2,4], L. MULLER[1,2,9];
[1]Applied Mathematics, [2]Brain and Mind Inst., [3]Biomed. Engin., [4]Clin. Neurolog. Sci., [5]Med. Imaging, [6]Med. Biophysics, [7]Psychology, [8]Epidemiology and Biostatistics, [9]Robarts Res. Inst., Western Univ., London, ON, Canada

Abstract: In the two-stage model of memory consolidation, memories are first formed in the hippocampus and then transferred to neocortex for long-term storage (McClelland et al., Psychological Review, 1995; Rasch and Born, Curr Opin Neurobiol, 2007). This transfer is mediated by synaptic plasticity, for which co-occurrence of pre- and post-synaptic activity plays a critical role (Bi and Poo, J Neurosci, 1998). While it has become increasingly clear that sleep oscillations actively and causally contribute to this process, it remains unclear to what extent these oscillations coordinate activity across areas in neocortex. Based on early recordings in animals under anesthesia, sleep rhythms were generally considered to occur across a wide range of cortex, creating a state of large-scale synchrony (Andersen et al., J Physiol, 1967; Contreras et al., J Neurosci, 1997; Achermann and Borbély, Neuroscience, 1998).
Recent work, however, has challenged this idea by reporting isolated sleep rhythms such as slow-oscillations and spindles. What is the spatial scale of sleep rhythms in cortex? To answer this question, we adapted deep learning algorithms initially developed for detecting earthquakes and gravitational waves in high-noise settings to analysis of neural recordings in sleep (Perol et

al., Science Advances, 2018; George and Huerta, Physics Letters, 2018). We studied sleep spindles in non-human primate electrocorticogram (ECoG), human electroencephalogram (EEG), and clinical intracranial electroencephalogram (iEEG) recordings. Our approach, which detects a range of clearly formed large- and small-amplitude spindles during sleep, reveals that sleep spindles co-occur over a broad range of cortex. In particular, multi-area spindles, where more than 10 electrode sites exhibit this rhythm simultaneously, are much more frequent than previously estimated by amplitude-thresholding approaches, which tend to select only the highest-amplitude spindles and can miss events that temporarily fall below threshold. Because spindles are intrinsically related to sleep-dependent consolidation of long-term memories, these results have important implications for the distribution of engrams in primate cortex.
We next hypothesized that, if multi-area spindles are actively involved in consolidating distributed networks in cortex, they may exhibit an increased prevalence across areas following a memory task. To address this additional hypothesis, we analyzed human EEG recordings following a low- and high-load visual working memory task. Consistent with this hypothesis, we find our method detects an increase in multi-area spindles in the high-load memory condition.

Disclosures: M. H. Mofrad: None. **G. Gilmore:** None. **S.M. Mirsattari:** None. **J.G. Burneo:** None. **D.A. Steven:** None. **A. Khan:** None. **A. Suller Mart:** None. **L. Muller:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.07

Topic: G.04. Emotion

Support: RF1AG061355

Title: The Dream Affect: The Role of Dreaming in Emotional Processing

Authors: *N. DELANO[1], A. PENA[1], K. SIMON[1], N. SATTARI[1], A. SHUSTER[1], E. ALZUETA[2], T. DULAI[2], M. DE ZAMBOTTI[2], F. BAKER[2], S. MEDNICK[1], J. ZHANG[1]; [1]Univ. of California Irvine, Irvine, CA; [2]SRI Intl., Menlo Park, CA

Abstract: Introduction Prior studies suggested that rapid eye movement sleep strengthens emotional memory while attenuating the affective tone (Werner, et al., 2015), and is a sleep stage where dreams are reported most often (Scarpelli et al., 2019). However, the role of dreaming in emotional processing remains unclear. Furthermore, dreaming experiences tend to be emotionally-valenced, but the effect of dream emotionality on emotional processing has been largely ignored. The goal of this study is to investigate the role of dreaming and its emotionality on sleep-dependent emotional processing. **Methods** 58 healthy women participated in this remote study (M_{Age}=36.24, SD=12.15). Participants completed the Emotional Picture Task

(EPT) encoding and immediate test before bedtime (Test 1). The next morning, participants reported their dreams through a sleep diary and were retested on the EPT (Test 2). During encoding, participants viewed negative and neutral images (selected from IAPS) and rated arousal (low to high) and valence (neutral to negative) for each image. During testing, participants reported if presented pictures were old or new and rated their arousal and valence. To examine whether dreaming influences emotional processing, we ran an independent sample t-test on EPT performance, valence and arousal ratings between dreamers and non-dreamers. We further divided dreamers into positive and negative based on subjective dream mood ratings to examine the effect of dreaming valence on emotional processing. **Results** 22 participants reported dreaming, 15 of which reported positive dreams. There was no significant difference between dreamers and non-dreamers on arousal/valence or memory performance ($p>.05$). Negative dreamers reported increased valence for negative images at Test 1 ($t(20)=2.16$, $p=.04$), higher arousal for negative (Test 1 $t(20)=2.81$, $p=.01$, Test 2 $t(20)=2.17$, $p=.04$) and neutral images (Test 1$t(20)=3.54$, $p<0.01$ Test 2 $t(20)=2.22$, $p=.04$) during both tests, compared to positive dreamers. Compared to non-dreamers, negative dreamers had increased arousal for negative images during both tests (Test 1:$t(41)=-2.97$, $p<.01$, Test 2: $t(41)=-2.12$, $p=.04$), and for neutral images during Test 1 ($t(41)=-2.58$, $p=.01$). **Conclusion** Our results suggest that the emotionality of dreams, rather than the act of dreaming, is implicated in emotional reactivity during wake. Specifically, those who have negative dreams tend to have higher levels of emotional reactivity. Our findings are inconsistent with the hypothesis that dreaming attenuates emotional reactivity. Instead, we found a continuity between dream emotion and waking emotional experiences.

Disclosures: N. Delano: None. **A. Pena:** None. **K. Simon:** None. **N. Sattari:** None. **A. Shuster:** None. **E. Alzueta:** None. **T. Dulai:** None. **M. de Zambotti:** None. **F. Baker:** None. **S. Mednick:** None. **J. Zhang:** None.

Poster

P813. Sleep and Memory Consolidation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P813.08

Topic: H.07. Long-Term Memory

Support: 5R35NS098224
F31MH123022

Title: Dopamine-based mechanism for transient forgetting

Authors: *J. SABANDAL, R. L. DAVIS;
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Abstract: Active forgetting is an essential component of the memory management system of the brain. Forgetting can be permanent, in which prior memory is lost completely, or transient, in which memory exists in a temporary state of impaired retrieval. Temporary blocks on memory seem to be universal, and can disrupt an individual's plans, social interactions and ability to make rapid, flexible and appropriate choices. However, the neurobiological mechanisms that cause transient forgetting are unknown. Here we identify a single dopamine neuron in Drosophila that mediates the memory suppression that results in transient forgetting. Artificially activating this neuron did not abolish the expression of long-term memory. Instead, it briefly suppressed memory retrieval, with the memory becoming accessible again over time. The dopamine neuron modulates memory retrieval by stimulating a unique dopamine receptor that is expressed in a restricted physical compartment of the axons of mushroom body neurons. This mechanism for transient forgetting is triggered by the presentation of interfering stimuli immediately before retrieval.

Disclosures: J. Sabandal: None. **R.L. Davis:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: American University Faculty Research Support Grant (AGZ),
Faculty Mellon Grant, NASA DC Space Grant
NIH 1R41NS113702-01 (AGZ),
SACP Pittcon Starter Grant, NSF I-Corps #1936173 (AGZ)
DC CFAR Pilot Award

Title: “Tyrosine and Oxytocin Detection with Carbon fiber-microelectrodes and fast scan cyclic voltammetry.”

Authors: *N. GHASEM ARDABILI[1], I. BROWN[2], C. COOK[2], F. LIU[2], A. G. ZESTOS[3]; [1]American Univ. DC, Wasington, DC; [2]American Univ., Washington DC, DC; [3]Chem., American Univ., Washington, DC

Abstract: “Tyrosine and Oxytocin Detection with Carbon fiber-microelectrodes and fast scancyclic voltammetry.”During the past few decades, carbon fiber microelectrodes (CFMEs) have been used forthe detection of biomolecules with fast scan cyclic voltammetry. This method has beendeveloped within the electrochemical field for improving analytical techniques such asmeasuring dopamine and other neurotransmitters. Peptides are vital and essential for the humanbody as they perform physiological and metabolic functions. Neurotransmitters, which are

one ofthe important classes of biomolecules, are important for determining several diseases andadministering pharmacological treatments. Oxytocin is a pleiotropic, peptide hormone that has awide impact on general health, adaptation, development, reproduction, and social behavior. Thisneuropeptide has different functions such as serving as a stress-coping molecule, anti-inflammatory agent, and serves as an antioxidant with protective effects especially in the face ofadversity or trauma.Moreover, this hormone can enhance social interactions, sexual activity, affiliative preferences,and parental behaviors. One of the important implications of oxytocin is in women's labor andfacilitates the procedure of parturition in women.With this method, we show the first measurement of Oxytocin with carbon electrodes andfast scan cyclic voltammetry. We applied a triangle waveform, when scanned from the holdingpotential of -0.2V to the switching potential of +1.2V and back. This waveform was insufficientand was not able to solely measure Oxytocin. Therefore, we developed the ModifiedWaveform(MSW), with a holding potential of -0.2V to the transition potential of +0.7V andswitching potential of +1.2V, with two different scan rates applied. Finally, we then measuredexogenously applied oxytocin on rat brain slices using the MSW waveform. This technique seeks
to develop novel assays for neuropeptide detection on a fast, subsecond timescale with possibleimplications for *in vivo* measurements.

Disclosures: N. Ghasem Ardabili: None. **I. Brown:** None. **C. Cook:** None. **F. Liu:** None. **A.G. Zestos:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: DC-CFAR
NIH 1R41NS113702-01
SACP Pittcon Starter Grant
NSF I-Corps #1936173
NSF MRI #1625977
American University Fall 2020 Graduate Student Research Grant
American University Faculty Research Support Grant

Title: Fscv and the novel carbon fiber multielectrode arrays for in vivo measurements of neurotransmitters

Authors: *H. RAFI[1], A. G. ZESTOS[2];
[2]Chem., [1]American Univ., Washington, DC

Abstract: FSCV, or fast scan cyclic voltammetry, is an electrochemical technique that measures the oxidation and reduction of various single molecules and proteins. They are oxidized/reduced at specific voltages which serve as a "molecular fingerprint" for detection. Carbon fiber microelectrodes (CFMEs) have been extensively used to measure neurotransmitters with FSCV due to their ability to adsorb cationic monoamine neurotransmitters. Although this method provides high temporal and spatial resolution, only single channel potentiostats and electrodes have been primarily utilized thus far. The need and use of carbon fiber multielectrode arrays has risen to target multiple brain regions. We characterized the novel carbon fiber multielectrode array (MEA), a four-channel electrode, and found it comparable to the single channel CFME. The MEA, when coupled with a commercially available multichannel potentiostat, had the additional capability of multiplexing neurotransmitter measurements *in-vitro* by multi-waveform application. The 4-channel potentiostat also allowed for the use and detection in multiple brain regions *ex-vivo*. In the present study we determined if the MEA could detect neurotransmitters endogenously when it was implanted *in-vivo* in anesthetized and freely behaving mice. Successful implantation of this electrode will aide in understanding complex brain heterogeneity, the dynamic neurochemical environment, and how disease states or drugs affect separate brain areas concurrently.

Disclosures: H. Rafi: None. **A.G. Zestos:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01NS116080

Title: All diamond microelectrodes for neurotransmitter measurement with fast-scan cyclic voltammetry

Authors: *J. R. SIEGENTHALER[1], R. RECHENBERG[1], A. HARDY[1], M. F. BECKER[1], E. K. PURCELL[2], W. LI[2];
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Abstract: Diamond is a versatile material that is biocompatible, chemically inert and has a wide potential window for electrochemical detection. Diamond has previously been grown on a tungsten wire and used to measure dopamine and other electroactive neurotransmitters both in vitro and in vivo with high spatial and temporal resolution using fast-scan cyclic voltammetry (FSCV). Here we report on the further development and optimization of an all diamond microelectrode that can electrochemically measure common neurotransmitters with a linear

response. The all-diamond microelectrode consists of a boron-doped polycrystalline diamond core encapsulated in a polycrystalline diamond insulating sheath improving on the biocompatibility, and flexibility in batch-wafer-fabrication. Using these electrodes, we characterized several common neurotransmitters for the linear dynamic range, detection limit and noise of the diamond electrodes, measuring dopamine, serotonin, 3,4-Dihydroxyphenylacetic acid, ascorbic acid, and hydrogen peroxide through FSCV. Using all diamond electrodes for neurotransmitter analysis is advantageous as it is the gateway to batch fabrication of microelectrodes, decreasing errors generated in the traditional hand fabrication methods, and building towards a scalable batch method for electrode array technologies.

Disclosures: J.R. Siegenthaler: None. **R. Rechenberg:** None. **A. Hardy:** None. **M.F. Becker:** None. **E.K. Purcell:** None. **W. Li:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH 1R41NS113702-01
NSF I-Corps #1936173
DC CFAR Pilot Award
NASA DC Space Grant
Faculty Mellon Grant
Pittcon SACP Starter Grant

Title: Multielectrode Arrays for Neurotransmitter Detection with Fast Scan Cyclic Voltammetry

Authors: A. G. ZESTOS[1], H. RAFI[2];
[1]Chem., [2]Neurosci., American Univ., Washington, DC

Abstract: The brain is by far the most heterogeneous organ, and it is critically important to monitor various brain regions simultaneously in order to understand complex pharmacological, drug, and behavioral states. Therefore, it is critically important to make high temporal resolution neurochemical measurements to study the phasic firing of neurons in several brain regions concurrently. Fast scan cyclic voltammetry (FSCV) and carbon-fiber microelectrodes (CFMEs) have been utilized used to detect several important neurochemicals *in vivo*. FSCV allows for high temporal measurements (< 100 msec) of neurotransmitter levels. Some of the many applications of this technique could be measuring neurochemical changes during epileptic seizures with simultaneous EEG measurements in rodents, drug abuse studies, measuring robust dopamine increases during deep brain stimulation in Parkinsonian models, and possibly many

others. This work will also discuss the development of multielectrode arrays (MEAs) for neurotransmitter detection with fast scan cyclic voltammetry in multiple brain regions simultaneously. Parylene and silicon insulated carbon fiber microelectrode arrays were shown to be able to measure neurochemicals in multiple brain regions simultaneously when coupled with multichannel potentiostats. Moreover, we have utilized techniques such as plasma enhanced chemical vapor deposition (PECVD) to deposit conductive carbon nanospikes onto the surface of existing metal multielectrode arrays to give them dual functionality as neurotransmitter sensors with FSCV in addition to being used primarily for electrical stimulation and recording. Other assays have shown the utility of electrodepositing carbon nanotubes and polymers such as PEDOT to coat metal arrays with carbon to give them dual sensing capabilities.

Disclosures: A.G. Zestos: None. **H. Rafi:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01NS099292
Arizona Biomedical Research Center Grant ABRC45952
NIH Grant P30 CA023074

Title: Induction of Episodic Headache Phenotypes in Sprague Dawley Rats following DAGLa Inhibition

Authors: *A. LEVINE[1], E. LIKTOR-BUSA[1], K. KARLAGE[1], L. A. GIANCOTTI[2], D. SALVEMINI[3], T. W. VANDERAH[1], T. M. LARGENT-MILNES[1];
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Abstract: OBJECTIVE This study examines the role of DAGL in headache development. We investigate the behavioral and physiologic changes associated with inhibition of DAGL in S/D rats.
BACKGROUND In two models of medication overuse headache, 2-AG levels were selectively depleted in the periaqueductal grey (PAG), suggesting distinct regulation of DAGL, the main biosynthetic enzyme for 2-AG, at times of headache pain. These results led to the hypothesis that reduction of 2-AG via blockade of DAGL would induce headache-like behaviors.
METHODS Inhibition of DAGL was performed via injection of non-selective DAGL (DH376, 10 mg/kg, IP) and selective DAGLα (LEI106, 20 mg/kg, IP) and DAGLβ (KT109, 20mg/kg, IP)

inhibitors. Following DAGL blockade, studies measured 2-AG and AEA levels via LC-MS as well as headache relevant behaviors, including periorbital and hind-paw allodynia, photophobia, anxiety-like behaviors, and the responsivity of induced pain behaviors to abortive antimigraine agents. Blood Brain Barrier (BBB) permeability following DAGLα inhibition was examined via an *in vitro* Trans-Endothelial Electrical Resistance (TEER) assay, followed by *in vivo* analysis of BBB permeability to radiolabeled sucrose.
RESULTS Injection of non-selective DAGL and selective DAGLα inhibitors induced facial sensitivity in 100% and ~60% of female rats, respectively, without induction of peripheral sensitivity. Induced periorbital allodynia was attenuated by administration of sumatriptan and olcegepant. Selective DAGLα inhibition induced significant photophobia, without anxiety-like behaviors or changes in voluntary movement. Analysis of AEA and 2-AG levels revealed reductions in 2-AG in the visual cortex and periaqueductal grey (PAG), without altering AEA. TEER analysis revealed significant decreases in endothelial resistance following DAGLα inhibition. Carotid artery perfusion with radiolabeled sucrose following DAGLα inhibition resulted in slight increase in C-14 sucrose permeation into CNS tissue.
DISCUSSION These results provide evidence for the induction of headache-like pain and light sensitivity without extracephalic allodynia, thus modeling the clinical presentation of episodic headache. DAGL inhibition was associated with increased permeability of the BBB, a physiologic change which has been correlated with migraine induction. DAGL inhibition, which reduces the time, cost, and invasiveness of currently accepted models of headache may fill the gap left by current models of headache that largely reflect manifestations of chronic migraineurs, and it may provide an avenue to study the transition from episodic to chronic headache.

Disclosures: A. Levine: None. **E. Liktor-Busa:** None. **K. Karlage:** None. **L.A. Giancotti:** None. **D. Salvemini:** None. **T.W. Vanderah:** None. **T.M. Largent-Milnes:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant 5R01AG050598

Title: Cerebral open flow microperfusion vs microdialysis: differences in neuronal damage and gliosis during measurement of hippocampal extracellular insulin

Authors: *S. DOUGLASS, A. R. TASSONE, J. REITANO, E. C. MCNAY;
Behavioural Neurosci., Univ. at Albany, Albany, NY

Abstract: Sampling the brain's extracellular space requires an invasive approach, which risks damaging the tissue and hence potentially confounding results. The most common technique, microdialysis (MD), has been shown to cause neuronal loss and gliosis immediately surrounding the probe and cannulation site, although appropriate timing of measurements can to some extent potentially mitigate this issue. An alternative to MD is cerebral open flow microperfusion (cOFM), which uses a thinner probe and an open-sided cannulae. Frontal cortex studies have suggested that cOFM may reduce gliosis and tissue damage in comparison to MD and may also better preserve blood-brain-barrier integrity during sampling. The absence of a dialysis membrane when using cOFM may also permit better recovery of some molecules: one such is insulin, which is (i) large compared to traditional MD analytes and (ii) potentially trapped by adhesion to the membrane. Insulin is an important target: we have shown that it regulates hippocampal metabolism and cognition, and loss of hippocampal insulin signaling is also a key factor in diseases such as Alzheimer's.
We hypothesized that cOFM would cause less tissue damage and might improve recovery of insulin during hippocampal sampling. 15-week-old Sprague Dawley were dual-cannulated with MD and cOFM cannulae, one per hippocampus, counter-balanced across animals. Female rats underwent estrous staging while males underwent a control procedure. After recovery from surgery, probes were inserted and samples taken for 90 min, after which rats were euthanized and perfused with paraformaldehyde, with brains harvested, processed, and sliced. Slices were analyzed for transmembrane protein 119 (TMEM-119) to label microglia, cluster of differentiation 68 (CD68) to label phagocytotic microglia, glial fibrillary acidic protein (GFAP) to label astrocytes, and NeuroTrace to label Nissl bodies in neurons. As a qualitative measure, slices were visually inspected for signs of hemorrhaging and tissue damage at the probe sites. Extracellular insulin was analyzed via ELISA, and extracellular glucose was analyzed via glucose oxidase assay. Our next steps are to build on these baseline measurements and optimized technology to measure fluctuations in hippocampal ECF insulin caused by diet and/or cognitive demand.

Disclosures: S. Douglass: None. **A.R. Tassone:** None. **J. Reitano:** None. **E.C. McNay:** None.

Poster

P910. Biochemical and Molecular Techniques III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P910.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Regulatory effects of TGF-β1 canonical and non-canonical pathways on leukocyte extravasation across brain endothelium in X-linked Adrenoleukodsytrophy

Authors: *S. K. MCCARTHY[1], A. C. BERENSON[2], C. A. LUSK[1], A. LENAIL[3], N. SASIDHARAN[4], P. L. MUSOLINO[1];
[1]Neurol., Massachusetts Gen. Hosp., Boston, MA; [2]Boston Univ., Boston, MA; [3]MIT, Boston, MA; [4]Ludwig Maximilian Univ. of Munich, Munich, Germany

Abstract: X-linked adrenoleukodystrophy (ALD) is a devastating neurologic disorder caused by mutations in the ATP binding cassette subfamily D member 1 (ABCD1) gene, a half transporter responsible for the import of VLCFA into peroxisomes. A devastating form of cerebral demyelination occurs in up to 60% of males following disruption of the blood-brain barrier (BBB) and migration of leukocytes into the white matter. Central to the permeability changes and leukocyte extravasation is the control of endothelial tight-junctions (TJs). Claudin 5 (CLN5) constitutes the main TJ protein in human cerebral white matter vessels and is dynamically regulated by activation of divergent transforming growth factor beta 1 (TGF-β1) signaling pathways. Loss of ABCD1 is known to downregulate CLN5 and increase TGF- β1 levels. Little is known about how increases in TGF-β1 alter CLN5 expression brain endothelium in ALD. Here we set to test the hypothesis that increased permeability of ABCD1-deficient brain endothelium is caused by downregulation of CLN5 in TJ via activation of the TGF-β1 canonical pathway, translocation of SMAD4 to the nucleus and binging to FOX01 in the silencer region of the CLN5 promoter. Following siRNA silencing of HBMECs we found increased phosphorylation of SMAD3 (p=0.0015). Conversely, FOXO1 showed significantly lower phosphorylation ratios after loss of ABCD1 (p=0.018). Using SIS3 compound, TGF-β1 canonical signaling is inhibited by suppressing SMAD3 phosphorylation without affecting the non-canonical AKT/PI3-kinase signaling pathways. This allowed us to test the interaction between canonical and non-canonical TGF-β1 pathway. Following inhibition of SMAD3 phosphorylation, we found an increase in CLN5 mRNA and protein levels in ABCD1 silenced (p=0.0243). We did not observe significant differences in the phosphorylation ratio of AKT (non-canonical pathway). Our findings thus, support our hypothesis that following loss of ABCD1 there is higher activation of the TGF- β canonical pathway compared with the non-canonical PI3K/AKT pathway leading to transcriptional downregulation of CLN5 by stabilization of FOXO1/SMAD4-p complex in its promoter. Further studies evaluating its functional consequences in-vitro and in-vivo are necessary to assess its potential as therapeutic target.

Disclosures: S.K. McCarthy: None. **A.C. Berenson:** None. **C.A. Lusk:** None. **A. Lenail:** None. **N. Sasidharan:** None. **P.L. Musolino:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.01

Topic: A.07. Developmental Disorders

Support: R01MH099660
R01DC015776

Title: Transcriptional regulation by Tbx1 of neonatal neural stem cells is a determinant for social behavior in mice

Authors: *T. HIRAMOTO[1], S. BOKU[2], G. KANG[1], S. ABE[2], M. B. E SILVA[4], M. NAGASHIMA[2], P. O'BROIN[4], K. YE[3], T. YAMAUCHI[1], T. V. MICHURIN[5], G. N. ENIKOLOPOV[5], N. HIROI[1,6,7];
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Abstract: The developmental origin, through which gene variants initiate cellular abnormalities and contribute to mental illness, is still poorly understood due to many technical constraints of human studies. Hemizygous deletion of human chromosome 22q11.2 is associated with social deficits of autism and schizophrenia. We found that constitutive heterozygosity of *Tbx1* impaired affiliative social communication and interaction and that Tbx1 protein was enriched in postnatal neural stem cells. We capitalize on this knowledge base to further identify the precise developmental time window, critical cell types and their functions and transcriptional target genes of Tbx1 in cell and mouse models. There were significantly fewer GFP-positive cells in the subgranular cell layer of the dorsal hippocampus of Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice, compared to Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/+}$ mice (N=5-6 mice per group), indicating that Tbx1 deficiency results in loss of postnatal/adult neural stem cells in the hippocampus. In culture of neonatal neural stem cells derived from neonatal hippocampus (N=4-6 cultures per group), Tbx1 protein expression peaked during the G1/S cycle shortly before the G2/M phase, indicating a Tbx1 surge precedes the onset of cell division in neonatal neural stem cells. *Tbx1* siRNA significantly reduced the rate of proliferation and the number of BrdU-positive neonatal neural stem cells without inducing apoptosis. When *Tbx1* heterozygosity was initiated in neonatal neural stem cells at postnatal days 1-5 (P1-5) in nestinCreERT;*Tbx1*$^{flox/+}$ mice (N=5-23 mice per group), it impaired social interaction without altering approach behaviors toward a novel object and social olfactory stimuli, an anxiety-related trait or motor activity. When tamoxifen was given at P21-25 (N=11-40 mice per group), it had no effect on social interaction. We next identified the transcriptional targets of Tbx1 in neonatal neural stem cells using ChIP-seq (N=7 clones). This analysis collectively identified 1,039 genes. Validation analyses identified *Pten* and *Tdo2* as Tbx1's functional target genes and transcriptional activity of *Pten* promoter was reduced by a deletion or mutations placed at its proximal 35 bp segment. Our data establish that *Tbx1* deficiency impairs social interaction via defective proliferation of neonatal stem cells and this gene is transcriptionally linked with *Pten* and other target genes, mutations of which are associated with autism, thereby revealing a new cellular substrate and molecular networks for

social interaction deficits associated with 22q11.2 hemizygous deletion, as well as TBX1 loss-of-function mutations.

Disclosures: T. Hiramoto: None. **S. Boku:** None. **G. Kang:** None. **S. Abe:** None. **M.B. e Silva:** None. **M. Nagashima:** None. **P. O'Broin:** None. **K. Ye:** None. **T. Yamauchi:** None. **T.V. Michurin:** None. **G.N. Enikolopov:** None. **N. Hiroi:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.02

Topic: A.07. Developmental Disorders

Title: Behavioral and neuroanatomical alterations in CNTNAP2 mouse model of autism spectrum disorder

Authors: *T. GANDHI, C. LEE;
Comparative Biomed. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Autism spectrum disorder (ASD) is comprised of a group of neurodevelopmental disorders characterized by social interaction and communication deficits and repetitive/restricted behaviors. In this study, we examined the behavioral and neuroanatomical alterations in a genetic mouse model of ASD, harboring a knockout of the contactin associated protein-like 2 gene (CNTNAP2-/-). One of the important neuropathological features in this mouse model of ASD is the abnormal developmental migration of neurons destined for superficial cortical layers. In our study, the laminar organization of somatosensory, auditory and visual cortical areas was analyzed by labeling with CUX-1, which is a marker for neurons normally localized to the superficial cortical layers (II-IV). Retrograde tract tracing was employed in order to map projections of these ectopic neurons. Finally, analysis of core behavioral domains, ultrasonic vocalization recordings in pups, grooming and social interaction tests, were conducted in adult mutant and control groups. By quantifying the laminar distribution of CUX-1 positive cells in cortical areas of this genetic model of ASD, we found more ectopic CUX-1 positive cells in lower layers (V and VI) of cortical areas as compared to the control animals. Some changes were observed in co-localized CUX-1-positive and fluorogold retrogradely labelled cells in lower layer 5 of primary cortical areas in the Cntnap2-/- mice compared to the WT mice. Furthermore, our results indicate reduced ultrasonic vocalizations in mutant mouse pups as compared to controls. Also, the mutant group exhibited a decrease in sociability and increased grooming in comparison to controls. Presence of ectopic neurons in the lower layer 5 of primary cortical areas may form normal (corticocortical connections) or altered connections (corticothalamic connections) with their respective targets. These results suggest that neuroanatomical alterations in cortical areas could account for some core autistic behaviors.

Disclosures: T. Gandhi: None. **C. Lee:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.03

Topic: A.07. Developmental Disorders

Support: NIH R01MH116002
Binational Science Foundation
Kavli Foundation
National Genetics Foundation
Simons Foundation
Spector Fund
Swebilius Foundation

Title: Characterization of the effects of autism risk gene chd8 mutation on zebrafish neurodevelopment

Authors: *S. E. FITZPATRICK[1], H. WEINSCHUTZ MENDES[2], T. CHEN[2], J. RIHEL[3], E. J. HOFFMAN[2];
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Abstract: Idiopathic autism spectrum disorder (ASD) is remarkably prevalent (1-2% of the population) and clinically complex. Despite decades of research, an understanding of the specific mechanisms underlying the abnormal neurodevelopment of ASD remains elusive. Whole genome and exome sequencing studies implicate over 100 genes as “autism risk genes” - genes in which de novo rare variants in offspring are sufficient to cause ASD. The majority of risk genes identified by these studies play a role in either regulation of gene expression or neuronal communication. Within the broad category of gene expression regulators are chromatin modifiers - genes that produce proteins involved in chromatin modification to affect the expression of downstream genes involved in neurodevelopment. One such chromatin modifier gene repeatedly identified by sequencing studies and highly associated with ASD is chromodomain-helicase DNA-binding protein 8 (*CHD8*). To study the effects of *chd8* mutation on early neurodevelopment, we generated a zebrafish line with a frameshift deletion in an early exon of *chd8* and performed behavioral, immunohistochemical, molecular, and gene expression analyses of 6 days post-fertilization (dpf) larvae to characterize the line. All experiments were performed using sibling homozygous, heterozygous, and wild-type mutants to avoid potential genetic background effects. Heterozygous and homozygous mutants survive to adulthood,

although homozygous mutants have decreased fertility and are grossly smaller as adults. Behavioral analyses show homozygous *chd8* mutants have decreased daytime activity and decreased total sleep at night despite an increased number of sleep bouts compared to wild-type siblings. Brain activity mapping using phosphorylated extracellular signal-related kinase (pERK) and total ERK staining shows differences in baseline neural activity. RNA sequencing suggests homozygous *chd8* mutants have alterations in several key neurodevelopmental pathways. This work suggests disrupting mutations in a master regulator gene have significant effects on zebrafish behavior and neurodevelopment. The *chd8* mutant zebrafish line will be useful for dissecting the molecular mechanisms that underlie these changes and provide insight into the altered neurodevelopment of autism.

Disclosures: S.E. Fitzpatrick: None. **H. Weinschutz Mendes:** None. **T. Chen:** None. **J. Rihel:** None. **E.J. Hoffman:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.04

Topic: A.07. Developmental Disorders

Support: NIH Grant: R01 NS101596
NIH Grant: U54 NS108874

Title: Development and description of Kcnq2 and Kcnq3 gain-of-function mice

Authors: *N. VARGHESE, K. SPRINGER, H. SOH, D. K. MULKEY, A. TZINGOUNIS; Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: A major development over the last few years is the recognition that gain-of-function (GOF) pathogenic variants in potassium channels could lead to neurological and neuropsychiatric disorders. This is particularly striking with KCNQ2 and KCNQ3 channels, where *KCNQ2* and *KCNQ3* GOF variants have been identified in patients with Developmental and Epileptic Encephalopathy (DEE) and more recently with Autism Spectrum Disorders (ASD) with or without epilepsy. Currently, the mechanisms by which *KCNQ2 and KCNQ3* GOF pathogenic variants lead to the various disorders is not fully known. This is partly due to the scarcity of mouse animal models carrying KCNQ2 and KCNQ3 GOF variants preventing a detail analysis of their impact in brain excitability. To fill this gap, we have developed constitutive $Kcnq3^{R231C}$ and conditional $Kcnq2^{R201C}$ knock-in mice to act as a foundation of our work and a resource to the neuroscience community. $Kcnq2^{R201C}$ and $Kcnq3^{R231C}$ are recurrent GOF human variants that stabilize the open state leading to constitutively open KCNQ2 and KCNQ3 channels at the physiological voltage range. Unlike

constitutive *Kcnq2*$^{R201C/+}$ heterozygous mice, constitutive heterozygous *Kcnq3*$^{R231C/+}$ mice survive to adulthood. To bypass the very early lethality of constitutive *Kcnq2*$^{R201C/+}$ mice, we developed conditional mice using a cre-mediated genetic switch approach (Kcnq2$^{fR201C/+}$), that is *Kcnq2*^{R201C} is expressed only in cells expressing cre-recombinase. In the absence of cre-recombinase *Kcnq2*$^{fR201C/+}$ animals survive to adulthood and no apparent behavioral abnormalities are seen. Crossing the Kcnq2$^{fR201C/+}$ mice with Emx1-cre mice allowed for expression of the *Kcnq2*^{R201C} to forebrain excitatory cells starting at ~E10-E11. These mice die starting at ~P40, suggesting that expression of *Kcnq2*^{R201C} in forebrain excitatory cells leads to premature lethality. We also found that expression of *Kcnq2*^{R201C} leads to hypoexcitable CA1 pyramidal neurons, whereas expression of *Kcnq3*$^{R231C/+}$ reduced excitability in only a subset of pyramidal neurons, segregated based on the radial axis. In summary, we have developed two new mouse KCNQ GOF mouse lines that could act as tools to understand how potassium channel GOF variants could alter network excitability in general.

Disclosures: N. Varghese: None. **K. Springer:** None. **H. Soh:** None. **D.K. Mulkey:** None. **A. Tzingounis:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.05

Topic: A.07. Developmental Disorders

Support: NIH/NIMH R01MH102603
Simons Foundation for Autism Research 573689
T32-HL139438 NHLBI Institutional Training Grant
NIH/NIMH 1F31NS117030-01

Title: The Role of Foxp1 and Foxp2 in Striatal D1 Spiny Projection Neurons

Authors: *N. AHMED, A. ANDERSON, K. SIVAPRAKASAM, G. KONOPKA;
Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Autism spectrum disorder (ASD) is diagnosed by impaired social communication and restrictive or repetitive behavior. Variants in the transcription factors FOXP1 and FOXP2 are amongst the most significant genes implicated in the development of ASD. Mutations in these genes have also been linked to expressive language impairments in patients, while mutations or knockout of either gene in mice has been implicated in ASD-like behavior. Both genes are co-expressed in the striatum, where they may work together to regulate gene expression. More specifically, both genes are expressed in the dopamine receptor expressing spiny projection neurons (SPNs) of the striatum, cell types that have enriched expression for ASD candidate

genes. Reduction or knockout of Foxp1 from the dopamine 2 receptor (D2) SPNs in mice results in behavioral, morphological, and physiological impairments with fewer changes upon deletion of Foxp1 from the dopamine 1 receptor (D1) SPNs. This difference may be due to the differential expression of Foxp1 and Foxp2 in the SPNs; Foxp1 is highly expressed in both D1 and D2 SPNs whereas Foxp2 is more highly expressed in the D1 SPNs relative to D2 SPNs. Taken together, we hypothesize that Foxp1 and Foxp2 have compensatory functions in the D1 SPNs. Utilizing mice that have a Drd1 specific knockout of Foxp1, Foxp2, or both, we have found that loss of both genes results in impaired motor learning, hypoactivity, and social behavior. Differential gene expression analysis from single nuclei RNA-sequencing implicates genes involved in ASD risk, locomotive behavior, and neuronal development and function. These data support the hypothesis that Foxp1 and Foxp2 functionally compensate for each other in D1 striatal neurons.

Disclosures: N. Ahmed: None. **A. Anderson:** None. **K. Sivaprakasam:** None. **G. Konopka:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.06

Topic: A.07. Developmental Disorders

Support: NIH Grant K01 NS116168
OHSU Fellowship for Diversity in Research

Title: The role of Pten during primary sensory neuron development

Authors: *A. FERNANDEZ, K. M. WRIGHT;
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Abstract: <Altered sensory processing is a common feature of autism spectrum disorders (ASD). The contribution of sensory processing centers in the brain to abnormal somatosensation in models of ASD is well established. Recent studies have also found that primary sensory neurons in the dorsal root ganglia (DRG) show abnormal function in *Mecp2* and *Gabrb3* animal models of ASD. Phosphatase and tensin homolog (*Pten*) is a known ASD susceptibility gene that functions as a key regulator of neuronal proliferation and differentiation pathways. We therefore examined how altered *Pten* signaling in primary sensory neurons affects peripheral nervous system circuit development. Using *Pten*hemizygous mice as a model for syndromic ASD, we determined that altered *Pten* signaling during development compromises primary sensory neuron population diversification. Loss of a single copy of *Pten* disrupts neuronal cell cycle regulation and primary sensory neuron progenitor differentiation, ultimately leading to altered proportions

of nociceptors and proprioceptors in adult mice. Using primary sensory neuron-specific *Cre* lines (*Isl1*^{Cre} and *Avil*^{CreERT2}) in combination with a conditional allele of *Pten* (*Pten*^{Flox}), we found that defects in population diversity result from cell-intrinsic alterations in *Pten* signaling during an early developmental window. Altogether, we have identified a critical role for *Pten* signaling in primary sensory neuron population diversification during development, and established the relationship between specific cell-intrinsic signaling mechanisms and peripheral nervous system organization. These findings offer insight into the contribution of the peripheral nervous system to a key clinical feature of ASD.>

Disclosures: A. Fernandez: None. **K.M. Wright:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.07

Topic: A.07. Developmental Disorders

Support: NIH Grant MH097949
Autism Speaks Grant 11857

Title: Disruption of mTORC1 rescues PTEN loss mediated neuronal hypertrophy

Authors: *K. TARIQ[1], A. CONCHING[1], E. CULLEN[2], S. A. GETZ[4], W. WANG[1], M. LI[1], M. C. WESTON[3], B. W. LUIKART[5];
[1]Dartmouth Col., Hanover, NH; [3]Neurolog. Sci., [2]Univ. of Vermont, Burlington, VT; [4]Geisel Sch. of Med. At Dartmouth, Lebanon, NH; [5]Mol. and Systems Biol., Dartmouth Col. Geisel Sch. of Med., Lebanon, NH

Abstract: Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid phosphatase that negatively regulates AKT/mTOR signaling pathway and serves as a brake on unregulated cellular growth. In a non-dividing neuronal cell population, PTEN inactivation leads to hypertrophy, and such neuropathology has been observed in individuals with Autism Spectrum Disorder (ASD). In mouse models of autism, conditional Pten knockout (KO) in hippocampus leads to larger soma size, dendritic hypertrophy, ectopic neuronal migration, and increased spine densities. The underlying molecular mechanisms producing such alterations in neuronal architecture remain incompletely understood. Using well-defined mouse genetic alleles and retroviral systems to specifically target developing granule neurons, we aimed to determine the role of mTORC1 (mammalian target of rapamycin complex 1) in the development of Pten-KO-mediated autism-associated hypertrophy. mTOR kinase regulates cell growth, proliferation, and survival by operating in two multi-protein complexes, one of them being mTORC1. For genetic disruption, we knocked out Raptor, which is a unique and obligatory component of

mTORC1. We found that disruption of mTORC1 completely rescued Pten-KO-mediated effects on soma size, migration, spine density, and dendritic arborization. Similarly for pharmacological inhibition of mTORC1, acute treatment with rapamycin was able to completely rescue Pten-KO-mediated effects on soma size, migration, spine density, and dendritic arborization. These results suggest Raptor as a potential therapeutic target for treatment of ASD.

Disclosures: K. Tariq: None. **A. Conching:** None. **E. Cullen:** None. **S.A. Getz:** None. **W. Wang:** None. **M. Li:** None. **M.C. Weston:** None. **B.W. Luikart:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.08

Topic: A.07. Developmental Disorders

Support: SFARI 400101
NARSAD 2017
ERC 802371
MSCA 845065
ERC 755816
SFARI 308939
NIH MH084164

Title: A cross-species link between mTOR-related synaptic pathology and functional hyperconnectivity in autism

Authors: *M. PAGANI[1], N. BARSOTTI[2], A. BERTERO[2], S. TRAKOSHIS[3], L. ULYSSE[4], A. LOCARNO[5], I. MISEVICIUTE[5], A. DE FELICE[1], C. CANELLA[1], K. SUPEKAR[6], A. GALBUSERA[1], V. MENON[6], R. TONINI[5], G. DECO[4], M. V. LOMBARDO[1], M. PASQUALETTI[2], A. GOZZI[1];
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Abstract: Postmortem studies have revealed increased density of excitatory synapses in the brains of individuals with autism, with a putative link to aberrant mTOR-dependent synaptic pruning. Autism is also characterized by atypical macroscale functional connectivity as measured with resting-state fMRI (rsfMRI). These observations raise the question of whether excess of synapses cause aberrant functional connectivity in autism. Using rsfMRI, electrophysiology and in silico modelling in Tsc2 haploinsufficient mice, we show that mTOR-dependent increased

spine density is associated with autism-like stereotypies and cortico-striatal hyperconnectivity. These deficits are completely rescued by pharmacological inhibition of mTOR. Notably, we further demonstrate that children with idiopathic autism exhibit analogous cortical-striatal hyperconnectivity, and document that this connectivity fingerprint is enriched for autism-dysregulated genes interacting with mTOR or TSC2. Finally, we show that the identified transcriptomic signature is predominantly expressed in a subset of children with autism, thereby defining a segregable autism subtype. Our findings causally link mTOR-related synaptic pathology to large-scale network aberrations, revealing a unifying multi-scale framework that mechanistically reconciles developmental synaptopathy and functional hyperconnectivity in autism.

Disclosures: M. Pagani: None. **N. Barsotti:** None. **A. Bertero:** None. **S. Trakoshis:** None. **L. Ulysse:** None. **A. Locarno:** None. **I. Miseviciute:** None. **A. De Felice:** None. **C. Canella:** None. **K. Supekar:** None. **A. Galbusera:** None. **V. Menon:** None. **R. Tonini:** None. **G. Deco:** None. **M.V. Lombardo:** None. **M. Pasqualetti:** None. **A. Gozzi:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.09

Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: Analysis of iba1 microglia activation in the hippocampus of adolescent NS-Pten knockout mice

Authors: *P. D. WOMBLE, K. BLANDIN, J. PILCHER, E. KWOK, G. SULLENS, D. S. COELHO, D. NGUYEN, J. N. LUGO, JR;
Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: The NS-*Pten* (neuron subset-specific) KO mouse model develops spontaneous seizures beginning as early as 6wks. PTEN is a negative regulator of the mTOR pathway and deletion results in hyper activation of the pathway. NS-*Pten* KO mice have morphological abnormalities, specifically to the granule layer of the dentate gyrus (dysplasia), an increase in soma size of neurons, and increases in neuroinflammation found through western blot assay. KO animals have shown deficits in behavioral tasks involved in sociability and learning and memory. This finding is not surprising given that there is a high comorbidity between epilepsy and other cognitive disorders such as Autism Spectrum Disorder (ASD). This current study utilized 4, 10, and 15-week-old wildtype (WT) and knockout (KO) mice. For immunofluorescence protocol, mice were perfused with 50mls of 1x PBS followed by 50mls of 4% PFA in PBS. Dorsal

hippocampus was located, and tissue was randomly sampled to obtain a total of 6 slices per animal. Slices were stained for Iba1 (microglia) and mounted using a mounting medium containing Dapi. For each animal, 3 of the 6 stained slices were imaged on either the left or right hemisphere then total number of microglia was averaged between them. Total number of Iba1 cells was counted in the CA1 region, CA2/3 region, dentate gyrus, and total hippocampus using a custom semi-automated counting system made by creating a program using macros from ImageJ (NIH). Preliminary findings show that across all groups there is a genotype difference that KO animals have an increased total number or Iba1 positive cells in their hippocampus. For 4-week-old mice, all regions of the hippocampus (CA1, CA2/3, dentate gyrus) of KO animals were found to have an increase in the number of microglia. For 10-week-old mice, KO animals had more microglia in the CA1, dentate gyrus compared to WT mice. For 15-week-old mice, only the dentate gyrus had an increase in the number of microglia. These data suggest an increase in immune reactivity in KO mice with hyperactive mTOR signaling in the hippocampus in an age-dependent manner. We found that Pten KO mice had an increase in the microglia marker Iba1 in all three time points. Additionally, we found that this increase was in all regions for the 4-week time point, only the CA1 and dentate gyrus for the 10-week time point, and only the dentate gyrus for the 15-week time point, suggesting that the dentate gyrus may play a critical role in the inflammatory response of the Pten KO mice. Further research is needed to look at other brain areas, as well as characterizing the different stages of microglia in Pten WT and KO mice.

Disclosures: P.D. Womble: None. **K. Blandin:** None. **J. Pilcher:** None. **E. Kwok:** None. **G. Sullens:** None. **D.S. Coelho:** None. **D. Nguyen:** None. **J.N. Lugo:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.10

Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776.

Title: Rapamycin Corrects Stereotypic and Social Behavior Deficits in NS-Pten Knockout Mice

Authors: *D. A. NARVAIZ, S. O. NOLAN-STRLE, G. SMITH, A. HOLLEY, M. VOLQUARDSEN, C. REYNOLDS, J. LUGO;
Baylor Univ., Waco, TX

Abstract: Genetic mutations to phosphatase and tensin homolog deleted on chromosome ten (*PTEN*) produces hyperactive mTOR signaling followed by neuronal hypertrophy and a progressive development of seizures. *PTEN* mutations are also thought to contribute to the development of intellectual disabilities and autistic behaviors. Rapamycin, an mTOR inhibitor,

has been shown to reverse neuronal hypertrophy and reduce seizures in neuronal subset specific *Pten* knockout (NS-*Pten* KO) mice. Although NS-*Pten* KO mice exhibit cognitive, social, and motor deficits, the impact of mTOR inhibition on the development of these behavioral impairments has not been determined in this model. NS-*Pten* KO and wild type (WT) male and female mice were administered 10 mg/kg of rapamycin for 10 days beginning at postnatal day 28. Treatment and control groups were then subjected to the following behavioral test battery to determine the impact of rapamycin on behavior: open field, elevated plus maze, marble burying, social chamber, and trace fear conditioning. Rapamycin improved social behavior, *p* < .05, and stereotypic behaviors during marble burying, *p* < .05, in all mice, as well as stereotypic behavior in the open field, *p* < .05 in KO mice. Across all mice, rapamycin impaired contextual fear memory, *p* < .05. There were no sex differences in either the behavioral phenotype or impact of rapamycin on behavior in NS-*Pten* KO mice. Contrary to previous analyses, we found NS-Pten KO mice displayed less exploratory behavior, *p* < .05, and more stereotypic behavior, *p* < .05, in the open field compared to WT mice, as well as increased freezing to baseline, *p* < .001, a marginal increase to freezing to contextual cues, and no differences in freezing to an auditory cue or during the intertrial interval during a trace fear conditioning task. These data show that administration of rapamycin reduces the impairment of stereotypic and social behavior deficits in NS-*Pten* KO mice but impairs contextual fear memory.

Disclosures: D.A. Narvaiz: None. **S.O. Nolan-Strle:** None. **G. Smith:** None. **A. Holley:** None. **M. Volquardsen:** None. **C. Reynolds:** None. **J. Lugo:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.11

Topic: A.07. Developmental Disorders

Support: NIH R15NS088776

Title: Early developmental impact of flurothyl seizures in FMR1 knockout mice

Authors: *K. BLANDIN[1], D. NARVAIZ[1], G. SULLENS[2], M. BINDER[4], A. FAUST[1], J. N. LUGO, JR[3];
[1]Psychology & Neurosci., Baylor Univ., WACO, TX; [2]Psychology & Neurosci., [3]Psychology and Neurosci., Baylor Univ., Waco, TX; [4]Yale Sch. of Med., Waco, TX

Abstract: Autism Spectrum Disorder (ASD) is characterized by changes in repetitive behaviors, social, and communication deficits. One of the leading causes of inherited intellectual disability and a significant genetic contributor to ASD is fragile x syndrome, which occurs through the

silencing of the FMR1 gene. There is also a strong relationship between the presence of seizures during early life and the development of autistic-like behaviors. In this study, we investigated the impact of superimposing several seizures on a genetic condition, to determine the impact of seizures, the impact of the genetic condition and the combined impact of both. In the present study we investigated the effects of several seizures over many days on the FMR1 knockout (KO) mice. On Postnatal days 7 to 11, we presented flurothyl-induced seizures to wildtype (WT) and FMR1 KO mice. The seizures occurred 3 times a day with two-hour interval between seizures, while the control group received no treatment. A battery of behavioral tests was performed in adult mice (postnatal day 90) to assess the effects of seizures early in life: nose poke, social partition and open field-tested hyperactivity, sociability, anxiety, repetitive and restrictive behavior. A two-way ANOVA test of our nose poke behavior task revealed that KO mice exhibited both increased consecutive nose pokes, $p<.05$ and a longer latency to first nose poke, $p<.05$. Seizure induction increased latency to first nose poke in KO mice, $p<.05$ compared to KO mice without seizures. In our social behavior task, we found that the mice with early-life seizures had a social behavior deficit compared to controls, $p<.05$. A two-way ANOVA of the open field task showed that seizures resulted in a decrease in vertical episode count, $p<.01$, stereotypy time, $p<.05$ and stereotypic episode count, $p<.01$. The KO mice displayed an increased number of clockwise revolutions compared to WT, $p<.05$. We replicated prior findings that FMR1 KO mice display deficits in repetitive behavior and a reduction in exploratory behavior. Early life seizures decreased sociability and stereotypy in WT and FMR1 KO mice. We did find that seizures superimposed on a genetic condition exacerbated the exploratory behavioral deficits. These results aid in understanding how we can use double-hit models to understand the relationship between a genetic condition and seizures during early life.

Disclosures: K. Blandin: None. **D. Narvaiz:** None. **G. Sullens:** None. **M. Binder:** None. **A. Faust:** None. **J.N. Lugo:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.12

Topic: A.07. Developmental Disorders

Support: NS088776

Title: Temporal response of hippocampal microglia to status epilepticus during the neonatal period in mice.

Authors: *D. SANTANA-COELHO, D. NARVAIZ, S. CHILUKURI, J. LUGO;
Baylor Univ., Waco, TX

Abstract: The developing brain is uniquely susceptible to seizures. Studies have demonstrated that seizures during the early postnatal period can have deleterious consequences to brain development leading to behavioral outcomes such as deficits in communication, cognition, and increased anxiety. However, the mechanisms by which neonatal seizures disrupt neurodevelopment are unclear. Microglia are resident brain immune cells that respond to insults and exhibit important roles in development such as synapse pruning, maturation, and function. Considering the detrimental roles of microglia during development, we aimed to assess microglial response to status epilepticus, seizures characterized by lasting longer than 30 minutes that commonly occur during the neonatal period in humans. To investigate the temporal response of microglia to status epilepticus, we treated mice with 2 mg/kg kainic acid (i.p.) at postnatal day 10. Following seizures, hippocampal microglia activation was assessed at 1, 2, 3, 4, and 5 days post seizures. Analysis of microglial activation was done using flow cytometry to quantify MHCII+ myeloid cells. Preliminary data demonstrated that microglial activation was significantly increased by status epilepticus ($p=0.0012$) with the highest activation at 1 and 2 days post-seizure. Full characterization of microglial response will enable us to identify a critical window where the use of therapeutic interventions might prevent the deleterious consequences that status epilepticus during early life can have on neurodevelopment.

Disclosures: D. Santana-Coelho: None. **D. Narvaiz:** None. **S. Chilukuri:** None. **J. Lugo:** None.

Poster

P044. Autism: Genetic Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P044.13

Topic: A.07. Developmental Disorders

Support: KCL departmental studentship ST10000

Title: Aggression and reward processing in Autism Spectrum Disorder

Authors: *A. HERTZ, N. BLACKWOOD, M. M. PETRINOVIC, G. MCALONAN; Forensic and Neurodevelopmental Sci., King's Col. London, Inst. of Psychiatry, Psychology, and Neurosci., London, United Kingdom

Abstract: Aggression is a common form of challenging behavior among individuals with autism spectrum disorder (ASD). Affecting as many as 2-out-of-3 individuals with ASD, aggression has serious detrimental effects. Aggression often exacerbates core ASD symptoms and is the strongest predictor of parental stress, yet we lack effective treatments. Thus, a neurobiologically informed approach to the understanding and treatment of aggression in ASD is needed. Therefore, this project is built upon 2 key pieces of evidence: i) aggression may have rewarding

properties, and ii) the reward system shows aberrant activity following social stimuli in individuals with ASD. We aim to investigate whether aggression associated with ASD may be a rewarding experience.
To this end, we used the *Neurexin1α* (*Nrxn1α*) knock-out (KO) mouse model as i) *Nrxn1α* is a category-1 classified ASD-linked gene and ii) because mutations in *Nrxn1α* were associated with aggression both in humans and animal models of ASD. Male *Nrxn1α* KO (n=15) mice and their Wild-Type (WT) (n=15) littermates were tested for sociability, aggression, and conditioned place preference (CPP) for aggression (where 1-out-of-2 contexts are paired with an aggressive encounter). Furthermore, activity of the brain reward circuitry following aggression was investigated by c-Fos immunohistochemistry and correlated with behavior.
Our results show that *Nrxn1α* KO mice exhibit increased aggression compared to their WT littermates. In contrast to WTs, *Nrxn1α* KO mice acquired a robust CPP to the aggression paired context, which was correlated with activity levels of several brain regions involved in reward processing.
Our study suggests that aggressive behavior in the *Nrxn1α* KO mice is associated with aberrant recruitment of the reward system.

Disclosures: A. Hertz: None. **N. Blackwood:** None. **M.M. Petrinovic:** None. **G. McAlonan:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.01

Topic: A.07. Developmental Disorders

Support: SFARI Pilot Grant

Title: The Differential Contribution of Excitatory and Inhibitory Neurons to Cortical Pathology in a Mouse Model of Autism Spectrum Disorder

Authors: *D. BOYLE[1], M. HANSON[1], D. NAGARAJAN[2], J. FITZGERALD[3], O. KOKIKO-COCHRAN[3], J. C. WESTER[2];
[1]The Ohio State Univ. Neurosci. Grad. Program, Columbus, OH; [2]Dept. of Neurosci., [3]Dept. of Neuroscience, Animal Behavior Core, The Ohio State Univ., Columbus, OH

Abstract: The Differential Contributions of Excitatory and Inhibitory Neurons to Cortical Pathology in a Mouse Model of Autism Spectrum Disorder Danielle Boyle, Meretta Hanson, Devipriyanka Nagarajan, Julie Fitzgerald, Olga Kokiko-Cochran and Jason Wester Autism Spectrum Disorder (ASD) is a neurodevelopmental disease considered among the most impactful in the United States. A primary hypothesis of the mechanism underlying ASD etiology is that the

ratio of excitation to inhibition (E/I) is imbalanced in the cortex, leading to disrupted information processing. E/I balance depends on the proper development of synaptic connections among subtypes of excitatory projection neurons (PNs) and inhibitory interneuron (INs). Thus, an imbalance in E/I may result from abnormal development of neurons from either population. *ARID1B* is a high confidence ASD risk gene that is initially expressed during early embryonic development in both excitatory PNs and inhibitory INs. Here, we used transgenic strategies in mice to conditionally knock out (cKO) one copy of *Arid1b* from either excitatory PNs or a subset of inhibitory INs (Parvalbumin+, PV+ and Somatostatin+, SST+). We used paired whole-cell patch clamp recording in acute cortical slices to investigate intrinsic membrane properties and synaptic physiology among mutant and control neurons. Our data show that cKO of *Arid1b* from PNs increases both their excitability and their rate of forming recurrent excitatory synaptic connections, which may contribute to E/I imbalance. In behavioral tests, conditional *Arid1b* haploinsufficiency in PNs led to disruptions in social novelty selectively in males. In contrast, cKO of *Arid1b* from INs does not alter the intrinsic membrane properties of either PV+ or SST+ INs, the rates at which they target neighboring PNs for synaptic connections, or the physiology of inhibitory synapses. However, the physiology of excitatory connections from non-mutant PNs to mutant PV+ and SST+ INs is altered. Specifically, the potency and success rate of excitatory postsynaptic currents (EPSCs) onto PV+ INs are enhanced. Furthermore, EPSCs recorded in SST+ mutant INs do not display synaptic facilitation in response to excitation from non-mutant PNs, which likely disrupts disynaptic inhibition and gating of PN dendritic plasticity. Collectively, our work demonstrates novel disruptions to synaptic physiology that could alter E/I balance in the cortex of *Arid1b* haploinsufficient mice.

Disclosures: D. Boyle: None. **M. Hanson:** None. **D. Nagarajan:** None. **J. Fitzgerald:** None. **O. Kokiko-Cochran:** None. **J.C. Wester:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.02

Topic: A.07. Developmental Disorders

Title: Neurodevelopmental disorder risk factors ADNP and POGZ affect convergent neuroinflammatory pathways

Authors: *M. E. CONROW-GRAHAM, J. B. WILLIAMS, J. A. MARTIN, P. ZHONG, B. A. REIN, Q. CAO, Z. YAN;
State Univ. of New York at Buffalo, Buffalo, NY

Abstract: ADNP and POGZ are two high-confidence risk factors for autism spectrum disorder (ASD) and intellectual disability (ID), but how haploinsufficiency of each gene is linked to these

neurodevelopmental disorders (NDD) is largely unknown. Using postmortem tissue from patients with ASD, we found diminished protein levels of ADNP and POGZ in the prefrontal cortex (PFC), a region highly implicated in NDD. To understand the functional role of these important risk factors, we investigated how Adnp or Pogz deficiency in PFC affects behavioral, transcriptomic, and synaptic function by using viral-based gene transfer to knock down *Adnp* and *Pogz* in PFC of male and female wild-type mice. Behavioral testing revealed impaired NDD-linked cognitive task performance with *Adnp*- or *Pogz*-deficiency. Patch-clamp recording showed impaired NMDA- and AMPA-mediated glutamatergic signaling in PFC pyramidal neurons from *Adnp*- or *Pogz*-deficient mice. Both ADNP and POGZ are chromatin remodelers, which could profoundly affect gene transcription in the brain. To determine whether their roles in gene transcription are linked to the behavioral and synaptic findings, we performed RNA-sequencing analysis. We found predominant transcriptional upregulation induced by *Adnp*- or *Pogz*-deficiency, with significant overlap in upregulated gene targets. Strikingly, 48% of commonly upregulated genes are involved in inflammation, as demonstrated by gene ontology and HUB gene analyses. In particular, many of these genes are part of the complement system, which is thought to affect synapse function and removal. Furthermore, immunohistochemistry reveals significant microgliosis and astrocytosis in PFC with ADNP or POGZ deficiency. These findings are reminiscent of multiple human findings of increased gliosis and pro-microglial signaling in NDD. Taken together, these data suggest that ADNP or POGZ deficiency leads to the elevation of inflammatory genes in the PFC, causing increased microglial activation and impaired synaptic transmission, which could contribute to the impairment of PFC-mediated cognitive behaviors. This study has provided novel insights into the convergent actions of two top risk factors for NDD and potential intervention targets.

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

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.03

Topic: A.07. Developmental Disorders

Support: Barbara Zucker Early Faculty Career Award

Title: In utero exposure to maternal Anti-Caspr2 antibodies leads to sustained microglial activation and impedes normal microglial maturation in male offspring

Authors: *B. SPIELMAN, D. CAMPBELL, C. BAGNALL-MOREAU, L. BRIMBERG; Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Exposure in utero to maternal anti-brain antibodies (IgG) has been associated with an increased risk of having a child with Autism Spectrum Disorder (ASD). Caspr2 protein (Contactin-associated protein-like 2, encoded by the ASD risk gene, CNTNAP2) is one of the most common targets of these maternal antibodies. We have shown that male, but not female mice born to dams immunized with the extracellular portion of Caspr2 show an ASD-like phenotype. Here, we examine the role of microglia in mediating the ASD-like phenotype in mice exposed in utero to anti-Caspr2 ("Anti-Caspr2") IgG or to adjuvants alone ("Control"). We observed increased microglial activation in E18.5 and adult Anti-Caspr2 male mice, corresponding to decreased dendritic arborization and reduced spines in the CA1 region of the hippocampus. Single nucleus (sn) RNA-seq of adult hippocampus from Anti-Caspr2 and Control male mice demonstrates that microglia may be halted in an immature, pruning stage in these mice. In adulthood, depletion and subsequent reconstitution of microglia using PLX3397, an inhibitor of colony stimulating factor 1 receptor, does not restore normal dendritic arborization nor does it change the activated phenotype of microglia. This suggests that in utero exposure to maternal anti-Caspr2 IgG affects microglial programming in males but not females, with male microglia arrested in an immature/pre-microglia stage, leading to sustained pruning. We hypothesized that early suppression of microglial activation using captopril, an FDA approved drug for hypertension and heart failure that has previously been shown to suppress microglial activation, will prevent the decreased dendritic arborization and reduced spines in the hippocampus that have been noted in Anti-Caspr2 male mice. This study is currently ongoing. Altogether, our model can be utilized to examine early sex differences in microglial activation in ASD and to assess microglia as targets for potential therapeutic strategies.

Disclosures: B. Spielman: None. **D. Campbell:** None. **C. Bagnall-Moreau:** None. **L. Brimberg:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.04

Topic: A.07. Developmental Disorders

Support: CIHR

Title: Enhancing potassium-chloride co-transporter-2 (KCC2) function in neurons by targeting protein-protein interactions

Authors: *V. A. RAVEENDRAN[1], J. C. PRESSEY[1], S. NIM[2], C. CORBI-VERGE[2], P. M. KIM[2], M. A. WOODIN[1];
[1]Cell and Systems Biol., [2]Terrence Donnelly Ctr. for Cell. & Biomolecular Res., Univ. of Toronto, Toronto, ON, Canada

Abstract: Fast synaptic inhibition in the adult brain is mediated by the neurotransmitter γ-aminobutyric acid (GABA). The hyperpolarizing action of GABA requires low intracellular chloride (Cl^-) which is maintained by the potassium-chloride co-transporter 2 (KCC2) in mature neurons. KCC2 protein expression and/or function can be regulated by its interactome, which can affect its ability to extrude Cl^- ions. Altered Cl^- homeostasis is associated with various neurological disorders including schizophrenia and autism spectrum disorder (ASD). Using two different approaches, we are investigating strategies to promote KCC2 function by targeting its interaction with novel interacting partners, namely Protein kinase C and casein kinase substrate in neurons protein 1 (PACSIN1) and 14-3-3. Our first approach used computational methods to develop peptide-based protein-protein interaction inhibitors (PPI inhibitors) which prevent KCC2-PACSIN1 interaction. PACSIN1 is a neuron-specific protein involved in vesicle transport and clathrin-mediated endocytosis, and has been identified as a negative regulator of KCC2. Therefore, inhibiting KCC2-PACSIN1 interaction using PPI inhibitors can provide a neuron-specific therapeutic strategy to rescue KCC2. I have identified and validated two PPI inhibitors that result in hyperpolarized E_{GABA} in primary neurons, indicating enhanced KCC2 function in the presence of these inhibitors. The second approach used genetic manipulation of the ε, γ, and θ isoforms of 14-3-3 which interact with KCC2 in co-immunoprecipitation assay. Overexpression of 14-3-3 γ isoform resulted in reduced KCC2 expression in primary neurons, revealing potential targets for the development of new PPI inhibitors. The remainder of this study will examine the mechanisms underlying regulation of KCC2 by these interacting partners and further development of PPI inhibitors to treat disrupted KCC2 function in neurological disorders such as ASD.

Disclosures: V.A. Raveendran: None. **J.C. Pressey:** None. **S. Nim:** None. **C. Corbi-Verge:** None. **P.M. Kim:** None. **M.A. Woodin:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.05

Topic: A.07. Developmental Disorders

Support: NINDS Grant NS096098 to KT
NINDS Grant 1F31NS113404-01A1 to SMB
ERA-NET Neuron (ADNPinMED) to IG
Drs. Ronith and Armand Stemmer and Arthur Gerbi (French Friends of Tel Aviv University) to IG
Anne and Alex Cohen (Canadian Friends of Tel Aviv University) to IG
NICHD Grant 1F31HD103405-01A1 to SAB

Title: Activity-dependent neuroprotective protein (adnp) promotes sex dependent cortical neuronal morphogenesis, connectivity, and calcium signaling.

Authors: *S. A. BENNISON[1], S. M. BLAZEJEWSKI[1], X. LIU[1], G. HACOHEN KLEIMAN[2], S. SRAGOVICH[2], S. ZOIDOU[3], O. TOULOUMI[3], N. GRIGORIADIS[3], I. GOZES[2], K. TOYO-OKA[1];
[1]Drexel Univ. Col. of Med., Philadelphia, PA; [2]Tel-Aviv Univ., Tel Aviv, Israel; [3]Aristotle Univ. of Thessaloniki, Thessaloniki, Greece

Abstract: Defective neuritogenesis has been strongly implicated in the etiology of many neurodevelopmental disorders, but a mechanistic understanding as to how and why neurite formation goes awry in disease states is yet to be understood. Single gene mutations in activity-dependent neuroprotective protein (ADNP) are among the most frequent among autism spectrum disorders (ASDs) and intellectual disability (ID), leading to the neurodevelopmental disorder ADNP syndrome. Symptoms of ADNP syndrome include ID, ASD, delayed speech and motor development, sleep disorder, and seizures. However, there are currently no approved treatment options for patients with mutations in ADNP, rendering a greater understanding of ADNP's functions during development and mutational etiology of high importance. Previous studies showed that during neuritogenesis, Adnp localizes to the cytoplasm/neurites, and *Adnp* knockdown inhibits neuritogenesis in culture. Here, we hypothesized that Adnp is shuttled from the nucleus to the cytoplasm during neurite formation and that this process is mediated by the known nuclear-cytoplasm protein re-localizer, 14-3-3. Indeed, applying the 14-3-3 inhibitor, difopein, blocked Adnp cytoplasmic localization. Furthermore, pull-down showed that Adnp bound the nuclear-cytoplasmic shuttle 14-3-3 and we identified phospho-dependent 14-3-3/Adnp binding sites mediated by PKC. Here, we discovered that knockdown of Adnp using *in utero* electroporation of mouse layer 2/3 pyramidal neurons in the developing somatosensory cortex led to previously unreported changes in neurite formation beginning at P0. Defects in pyramidal neurons were sustained throughout development, the most notable including increased basal dendrite number, increased axon length, and increased excitatory axon innervation to different cortical areas. Paralleling the observed morphological aberrations, *ex vivo* calcium imaging revealed that Adnp deficient neurons had greater and more frequent spontaneous calcium influx in female mice. GRAPHIC, a novel synaptic tracing technology, substantiated this finding, revealing increased interhemispheric connectivity between female Adnp deficient layer 2/3 pyramidal neurons in the somatosensory cortex. Furthermore, in the Adnp syndrome mouse model we found markers of excitotoxicity in the female cortex. We conclude that Adnp is shuttled to the cytoplasm by 14-3-3, where it regulates neurite formation, maturation, and functional cortical connectivity significantly building on our current understanding of Adnp function and the etiology of ADNP syndrome.

Disclosures: S.A. Bennison: None. **S.M. Blazejewski:** None. **X. Liu:** None. **G. Hacohen Kleiman:** None. **S. Sragovich:** None. **S. Zoidou:** None. **O. Touloumi:** None. **N. Grigoriadis:** None. **I. Gozes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NAP (CP201) use is under patent protection (US patent nos. US7960334, US8618043, and US10912819), Ramot at Tel Aviv University.. **K. Toyo-oka:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.06

Topic: A.07. Developmental Disorders

Support: NIH Grant ES031050
L. S. Skaggs Presidential Endowed Chair

Title: Top2a promotes the development of social behavior via PRC2 and H3K27me3

Authors: *Y. GENG[1], T. ZHANG[1], S. GODAR[1], B. PLUIMER[2], D. HARRISON[3], A. NATH[4], J.-R. YEH[5], I. DRUMMOND[6], M. BORTOLATO[1], R. PETERSON[1];
[1]Univ. of Utah, SALT LAKE CITY, UT; [2]USC, Los Angeles, CA; [3]Univ. of Chicago, Chicago, IL; [4]Beth Israel Deaconess Med. Ctr., Boston, MA; [5]Massachusetts Gen. Hosp., Boston, MA; [6]MDI Biol. Lab., Bar Harbor, ME

Abstract: Human infants exhibit innate social behaviors at birth, a trait that is closely associated with neurodevelopmental disorders such as autism spectrum disorder, yet little is understood about the embryonic development of sociality. Using an automated high-throughput behavioral analysis system which we created and named Fishbook, we screened 1120 known drugs and found that embryonic inhibition of topoisomerase IIα (Top2a) resulted in lasting social deficits in zebrafish. In mice, prenatal Top2 inhibition caused behavioral defects related to core symptoms of autism, including social communication impairments and increased perseveration. Several known or suspected environmental risk factors of autism are Top2 inhibitors, indicating that Top2 inhibition may be a common mechanism through which environmental insults contribute to the development of autism. Mutation of Top2a in zebrafish caused downregulation of a set of genes highly enriched for genes associated with autism in humans. Both the Top2a-regulated genes and known autism-associated gene possess binding sites for polycomb repressive complex 2 (PRC2), a regulatory complex responsible for H3K27 trimethylation. Moreover, both gene sets are highly enriched for H3K27me3. Strikingly, inhibition of PRC2 component Ezh2 rescued the social deficit caused by Top2 inhibition. Therefore, Top2a is a key component of an evolutionarily conserved pathway that promotes the development of social behavior through PRC2 and H3K27me3. For future studies, the Fishbook platform enables systematic evaluation of environmental chemicals to identify those affecting social behavior development.

Disclosures: Y. Geng: None. **T. Zhang:** None. **S. Godar:** None. **B. Pluimer:** None. **D. Harrison:** None. **A. Nath:** None. **J. Yeh:** None. **I. Drummond:** None. **M. Bortolato:** None. **R. Peterson:** None.

Poster

P050. Autism: Physiology and Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P050.07

Topic: A.07. Developmental Disorders

Support: Independent Investigator Award (GMM) from the Brain and Behaviour Research Foundation
Support from Clinical Research Associates, L.L.C. (CRA), an affiliate of the Simons Foundation
Support from Sackler Institute for Translational Neurodevelopment at King's College London
EU-AIMS (European Autism Interventions)/EU AIMS-2-TRIALS, an Innovative Medicines Initiative Joint Undertaking under Grant Agreement No. 777394
Support from National Institute for Health Research (NIHR) Mental Health Biomedical Research Centre (BRC) at South London and Maudsley NHS Foundation Trust and King's College London

Title: The aperiodic 1/f signal as a proxy marker of excitation- inhibition flux in the brain: evidence from individuals with and without autism

Authors: *C. ELLIS[1], J. AHMAD[2], A. ZOUMPOULAKI[3], H. E. VELTHUIS[1], A. PEREIRA[1], N. M. L. WONG[4], M. DIMITROV[1], M. F. PONTEDURO[1], L. KOWALEWSKI[1], A. LEONARD[5], E. MCGREGOR[6], P. GARCES[7], Q. HUANG[1], E. DALY[1], D. MURPHY[1], G. MCALONAN[1];
[1]King's Col. London, London, United Kingdom; [2]Univ. of Greenwich, London, United Kingdom; [3]Cardiff Univ., Cardiff, United Kingdom; [4]The Univ. of Hong Kong, Hong Kong, Hong Kong; [5]Royal Col. of Surgeons in Ireland, Dublin, Ireland; [6]Univ. of East London, London, United Kingdom; [7]Ho, Basel, Switzerland

Abstract: Efficient brain function is governed by coordination of Excitatory (E) glutamate and Inhibitory (I) GABA signalling. E-I regulation is thought to be disrupted in neurodevelopmental conditions such as autism. However, in living humans, investigation of E-I is constrained by the methods available which lack temporal resolution (Magnetic Resonance Spectroscopy) or are invasive (Positron Emission Tomography). Hence, as E-I signalling is dynamic, complementary metrics are needed to track its flux in real time. Electroencephalography (EEG) has good temporal resolution suited to this goal. Computational modelling and animal studies suggest the aperiodic exponent of the EEG power spectrum may be a proxy of E-I, where more inhibition is associated with larger exponents. However, whether this relationship translates to typical humans is unknown, let alone in autism. In this Proof of Concept (PoC) study we hypothesised that the aperiodic exponent is sensitive to E-I pharmacological challenge using a GABAergic agonist, arbaclofen, in autistic and non-autistic individuals. Participants were 33 adults, 18 with autism.

EEG was recorded during an auditory odd-ball paradigm after placebo and after 30mg of arbaclofen. Data were segmented into 2.4 seconds of 4 standard tones. Power Spectral Densities were extracted and parameterised using the FOOOF model to separate periodic (oscillatory) from aperiodic activity. A 2 x 2 mixed ANOVA was performed with dose as the within subjects' factor (placebo or 30mg), group as the between subjects' factor (autism or no-autism) and aperiodic exponent as the dependent variable. On average, aperiodic exponents from autistic individuals (M = 1.27, SD = 0.25) were smaller than those from non-autistic individuals (M = 1.41, SD = 0.23); but, this did not reach statistical significance ($F(1, 31) = 3.23$, $p = 0.082$). However, there was a significant main effect of drug dose $F(1, 31) = 4.44$, $p = 0.043$, whereby aperiodic exponents after 30mg of arbaclofen (M = 1.37, SD = 0.27) were larger than those at placebo (M = 1.30, SD = 0.24) across both groups. There was no significant interaction between dose and group on aperiodic exponent; $F(1, 31) = 1.77$, $p = .194$. We provide the first PoC evidence that, in humans, the aperiodic exponent is sensitive to GABAergic drug challenge and so may be a proxy marker of E-I. Although not statistically significant, the lower aperiodic exponent in the autism group compared to neurotypicals at placebo was increased by arbaclofen. The next steps will be to determine if shift in E-I (as captured by aperiodic shift) predicts a clinical response to a potential pharmacological intervention (with arbaclofen) in autism at an individual level.

Disclosures: C. Ellis: None. **J. Ahmad:** None. **A. Zoumpoulaki:** None. **H.E. Velthuis:** None. **A. Pereira:** None. **N.M.L. Wong:** None. **M. Dimitrov:** None. **M.F. Ponteduro:** None. **L. Kowalewski:** None. **A. Leonard:** None. **E. McGregor:** None. **P. Garces:** A. Employment/Salary (full or part-time):; F. Ho. **Q. Huang:** None. **E. Daly:** 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.; Industry collaborations within AIMS-2-TRIALS network. **D. Murphy:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support from EFPIA to EU IMI AIMS-2 TRIALS. F. Consulting Fees (e.g., advisory boards); Roche, Servier, and Jaguar Theraputics. **G. McAlonan:** 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.; PI for an investigator initiated study funded by industry and industry collaborations within AIMS-2-TRIALS Network. F. Consulting Fees (e.g., advisory boards); Scientific Advisory Board Greenwich Pharmaceuticals.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.01

Topic: A.07. Developmental Disorders

Support: Diversity Supplement for R01GM046779 NIGMS
T32 Medical Scientist Training Program (MSTP)

Title: Implications of FMRP Control of Ribosome Translocation

Authors: *M. A. NASRALLAH, S. JUNG, S. SHAH, O. AYDEMIR, J. D. RICHTER;
Mol. Med., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Fragile X Syndrome (FXS) is a neurodevelopmental disorder (NDD) lying on the autism spectrum whereby afflicted individuals display developmental delays, learning impairment, hyperexcitability, epilepsy, and non-neurological maladies. FXS is caused by a triplet repeat expansion in the *FMR1* gene, which leads to its DNA methylation and transcriptional inactivation. *FMR1* encodes Fragile X Mental Retardation Protein (FMRP), an RNA binding protein (RBP) that regulates mRNA translation. In the mouse brain, one mechanism by which FMRP inhibits protein synthesis is by stalling ribosome translocation along mRNA, thereby slowing polypeptide elongation. In the FMRP knockout (KO) brain, ribosome translocation is rapid, thereby leading to elevated translation of specific mRNAs, many of which are implicated in autism. Our lab recently reported that FMRP stalls ribosome translocation on specific mRNAs in the mouse hippocampus, particularly on those that establish epigenetic marks on chromatin (Shah/Molinaro et al 2020). However, how FMRP controls ribosome translocation on specific mRNAs remains elusive. Here, we used CRISPR-Cas 9 edited murine neuroblastoma (mN2a) cells to generate a deletion in *Fmr1* exon 3 that prevents FMRP expression. We probed the dynamics of ribosome translocation through run-off polysome sequencing from WT and FMRP-deficient mN2a cells. Our preliminary results confirm that FMRP associates with stalled polysomes in mN2a cells and reveal, on a whole transcriptome level, that FMRP stalls ribosome translocation on specific mRNAs in this cell type. We aim to perform further mechanistic analyses such as run-off ribosome profiling and genetic loss-of-function experiments in various RNA-binding domains in FMRP to define the region governing its function of ribosome stalling. Additionally, we aim to perform similar analyses in WT and *Fmr1* KO mouse hippocampal slices. These studies will improve our understanding of the molecular basis of FXS and other autism spectrum disorders (ASDs). Shah, S., Molinaro, G., Liu, B., Wang, R., Huber, K.M., and Richter, J.D. (2020). FMRP Control of Ribosome Translocation Promotes Chromatin Modifications and Alternative Splicing of Neuronal Genes Linked to Autism. Cell Rep. *30*, 4459-4472.e6.

Disclosures: M.A. Nasrallah: None. **S. Jung:** None. **S. Shah:** None. **O. Aydemir:** None. **J.D. Richter:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.02

Topic: A.07. Developmental Disorders

Support: Jim and Betty Ann Rodgers Chair Fund
Council on Creative Research FSU

Title: Comparing of the Effects of Two Kappa Opioid Receptor Antagonists in male and female Fragile X transgenic mice

Authors: D. M. MCCARTHY, A. J. CANEKERATNE, O. G. MOORE, N. E. GILLEN, P. G. BHIDE;
Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Fragile X syndrome (FXS) is the number one inherited cause of intellectual disabilities, and the most common genetic cause of autism spectrum disorder. Neuro-behavioral symptoms of FXS include anxiety, attention deficit, hyperactivity, and changes in social interactions consistent with autistic behavior. FXS is caused by inactivation of the X-linked fragile-X mental retardation -1 (*FMR1*) gene. Despite intensive preclinical and clinical research, effective treatment for FXS is not available at this time. The *Fmr1* knockout (KO) mouse, in which the *Fmr1* gene is deleted is an excellent model to evaluate potential novel treatments for FXS. The mouse model displays behavioral impairments that are consistent with the behavioral symptoms reported in FXS. We showed that *Fmr1* KO male and female mice have significant impairment in nest building behavior, which is an excellent proxy for compulsive behavior and social behavior. Since activation of the kappa opioid receptor (KOR) disrupts nest building in wild type mice, we hypothesized that KOR antagonists improve nest building in the *Fmr1* KO mouse. To test our hypothesis, we examined the effects of two kappa opioid receptor antagonists, norbinaltorphimine (NorBNI) and aticaprant (LY245630) on nest building behavior in *Fmr1* KO male and female mice. Although both the compounds are selective KOR antagonists, the effects of norBNI last significantly longer than those of LY245630, likely because each compound activates a distinct intracellular signaling cascades. A single intraperitoneal administration of norBNI (20mg/kg) or LY245630 (10mg/kg) to *Fmr1* KO male mice improved nest building. However, the effects of norBNI lasted for up to 3 weeks whereas those of LY245630 lasted only up to 24 hours. No significant improvement was seen in female *Fmr1* KO mice with either compound at the doses used here, suggesting sex-differences in the response to KOR antagonists. We are examining the potential cellular and molecular mechanisms underlying the sex differences. Collectively, our data suggest that the KOR plays a role in nest building behavior in the *Fmr1* KO mouse and that FXS drug development efforts could target the KOR.

Disclosures: D.M. McCarthy: None. **A.J. Canekeratne:** None. **O.G. Moore:** None. **N.E. Gillen:** None. **P.G. Bhide:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.03

Topic: A.07. Developmental Disorders

Support: NIH grant R01MH116582
NIH grant R01NS105200
NIH grant R01MH078972
NIH grant R21NS098767
NIH grant R56MH113146
NIH grant R21HD085288
NIH grant R03HD083538

Title: Identification of FMR1 regulated molecular networks in human neurodevelopment

Authors: M. LI, J. SHIN, R. RISGAARD, M. PARRIES, J. WANG, D. CHASMAN, S. LIU, S. ROY, X. ZHAO, ***A. BHATTACHARYYA**;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: RNA binding proteins (RNA-BPs) play critical roles in development and disease to regulate gene expression. However, genome-wide identification of their targets in primary human cells has been challenging. Here, we applied a modified CLIP-seq strategy to identify genome-wide targets of the FMRP translational regulator 1 (FMR1), a brain-enriched RNA-BP, whose deficiency leads to Fragile X Syndrome (FXS), the most prevalent inherited intellectual disability. We identified FMR1 targets in human dorsal and ventral forebrain neural progenitors and excitatory and inhibitory neurons differentiated from human pluripotent stem cells. In parallel, we measured the transcriptomes of the same four cell types upon FMR1 gene deletion. We discovered that FMR1 preferentially binds long transcripts in human neural cells. FMR1 targets include genes unique to human neural cells and associated with clinical phenotypes of FXS and autism. Integrative network analysis using graph diffusion and multi-task clustering of FMR1 CLIP-seq and transcriptional targets reveals critical pathways regulated by FMR1 in human neural development. Our results demonstrate that FMR1 regulates a common set of targets among different neural cell types, but also operates in a cell type-specific manner targeting distinct sets of genes in human excitatory and inhibitory neural progenitors and neurons. By defining molecular subnetworks and validating specific high priority genes, we identify novel components of the FMR1 regulation program. Our results provide new insights into gene regulation by a critical neuronal RNA-BP in human neurodevelopment.

Disclosures: M. Li: None. **J. Shin:** None. **R. Risgaard:** None. **M. Parries:** None. **J. Wang:** None. **D. Chasman:** None. **S. Liu:** None. **S. Roy:** None. **X. Zhao:** None. **A. Bhattacharyya:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.04

Topic: A.07. Developmental Disorders

Support: R01NS109381

Title: Astrocytic contribution to audiogenic seizures in a mouse model of fragile X syndrome

Authors: *L. BERGDOLT[1], P. RAGUNATHAN[2], M. DOUCHEY[2], A. REID[2], E. ELDRIDGE[2], O. TARASCHENKO[2], A. DUNAEVSKY[2];
[2]Dept. of Neurolog. Sci., [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fragile X syndrome (FXS) is caused by the silencing of the fragile X mental retardation protein (FMRP). Patients with FXS are often also diagnosed with autism spectrum disorder and exhibit additional symptoms which may include intellectual disability, seizures, anxiety, and sensory hypersensitivity. FMRP, encoded by the *Fmr1* gene, is known to regulate protein expression via interactions with mRNA or protein. Despite extensive characterization of FMRP function, particularly in neurons, the effects of FMRP deletion on astrocyte physiology and the contribution of astrocytes to FXS symptoms, particularly sensory hypersensitivity, remain understudied. A robust phenotype in *Fmr1* knockout (KO) mice is susceptibility to audiogenic seizures, manifested as wild running and tonic-clonic seizures following exposure to a 120 dB siren. Using the inducible cre line Aldh1l1-cre/ERT2, we have found that astrocyte-specific deletion of *Fmr1* (*Fmr1* conditional KO) during the first postnatal week confers susceptibility to audiogenic seizures in three-week-old male mice. Moreover, selective restoration of *Fmr1* expression in astrocytes decreases the propensity for audiogenic seizures. Six-week-old *Fmr1* conditional KO mice do not exhibit observable seizure behavior in response to the auditory stimulus, but we have found a higher propensity for seizure activity detected by electroencephalogram recordings. Taken together, these data suggest that astrocytes play a role in auditory hypersensitivity and hyperexcitability in *Fmr1* KO mice. A key way astrocytes could contribute to altered neuronal signaling is through alterations in intracellular calcium signaling. Indeed, in acute brain slices from mice expressing the genetically encoded calcium indicator GCaMP6f, we have found enhanced spontaneous and ATP-induced somatic calcium signaling in cortical astrocytes in *Fmr1* KO mice. Overexpression of the purinergic receptor P2Y1R may contribute to this phenotype, as we have found a significant increase in P2Y1R expression in the cortex of *Fmr1* KO mice. Furthermore, enhanced calcium signaling is normalized by pretreatment with a P2Y1R antagonist. *Fmr1* knockout mice also exhibit enhanced calcium signaling in astrocytic processes *in vivo*. We also observed a smaller enhancement of ATP-induced calcium signaling in acute brain slices from *Fmr1* conditional KO mice Together, these data suggest that the mechanisms underlying elevated calcium signaling in *Fmr1* knockout mice

are both intrinsic and extrinsic. Ongoing experiments examine if altered astrocytic calcium signaling contributes to sensory hypersensitivity in FXS.

Disclosures: L. Bergdolt: None. **P. Ragunathan:** None. **M. Douchey:** None. **A. Reid:** None. **E. Eldridge:** None. **O. Taraschenko:** None. **A. Dunaevsky:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.05

Topic: A.07. Developmental Disorders

Title: Abnormal spine development alters visual responses in a zebrafish model of Fragile X syndrome

Authors: *I. S. Y. WONG, E. C. DEMARCO, A. LAMARCA DELA PLATA, E. ROBLES; Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN

Abstract: Fragile X syndrome is caused by loss-of-function mutations in the *fmr1* gene, which encodes Fragile-X Mental Retardation protein, a translational regulator highly expressed in the brain. At the level of individual neurons, loss of *fmr1* results in abnormal development of dendritic spines, the principal postsynaptic site of excitatory synaptic transmission in mammalian cortex. To non-invasively monitor spine development in vivo, we combined an *id2b:gal4* transgenic zebrafish line with mosaic genetic labeling to specifically label single pyramidal neurons (PyrNs) in the optic tectum. Live imaging of PyrN dendritic morphology during larval development revealed rapid formation of dendritic spines between 4- and 8-days postfertilization (dpf). At 4 dpf, PyrN dendrites have long filopodia-like protrusions with high turnover rates, whereas stable spine-like protrusions with enlarged heads predominate at 8 dpf. To determine if PyrN spine development is affected by the loss of *fmr1*, we examined PyrN morphology in homozygous *fmr1* mutants. Live imaging of single neurons in *fmr1* mutants revealed a reduction in dendritic area and length. At the level of spines, loss of *fmr1* caused a significant decrease in spine number and spine stability. These findings support a synaptotrophic model of dendrite growth in which *fmr1* normally functions to promote synapse formation and spine stabilization, which in turn positively influences dendritic growth. Next, we sought to determine if the reduction in spines caused by loss of *fmr1* leads to altered visual responses in PyrNs. Response properties in WT and *fmr1* larvae were assessed by monitoring GCaMP6s fluorescence in PyrNs during presentation of a luminance ramp stimulus. These experiments revealed altered response kinetics in PyrNs lacking *fmr1* during early larval development (3 and 4 days), although these changes were not evident in older larvae (6 or 8 dpf). This suggest that loss of *fmr1* may cause a delay in the formation of synaptic inputs that shape PyrN responses to luminance ramps. Experiments are currently underway to determine if the receptive fields (RFs) of PyrNs are

altered in *fmr1* mutants. Preliminary data indicate that WT PyrNs have large RFs that span a large portion of the retinal hemifield, whereas PyrNs in *fmr1*-/- larvae exhibit significantly reduced RF sizes. Together these data suggest that the formation of stable spines on PyrN dendrites is necessary for the generation of large visual RFs. Overall, these findings establish the zebrafish PyrN system as a valuable model system to quantify spine defects in vivo and to assess the functional consequences.

Disclosures: I.S.Y. Wong: None. **E.C. DeMarco:** None. **A. Lamarca Dela Plata:** None. **E. Robles:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.06

Topic: A.07. Developmental Disorders

Support: R01MH106469
FRAXA
T32MH112510

Title: Protein synthesis-dependent hyperexcitability of Fmr1-KO visual cortex layer 5 pyramidal cells offers path to identify novel therapeutic targets for the treatment of fragile X syndrome

Authors: *M. J. HEINRICH, D. C. STOPPEL, F.-J. WENG, P. K. MCCAMPHILL, R. K. SENTER, M. F. BEAR;
The Picower Inst. for Learning & Memory, MIT, Cambridge, MA

Abstract: Caused by the silencing of the *FMR1* gene encoding fragile X mental retardation protein (FMRP), fragile X syndrome (FXS) in humans has been modeled in *Fmr1*-knockout (KO) mice. It is now recognized that there are different classes of pathophysiological mechanisms in the *Fmr1*-KO, one of which is altered regulation of neuronal protein synthesis. Within this class, a subset of FXS phenotypes respond immediately to inhibition of protein synthesis, suggesting involvement of pathogenic proteins that are rapidly turned over. Identifying these proteins, and linking them directly to altered neuronal physiology that could be used as biomarkers, is the goal of this project. To achieve this objective, one must identify an accessible population of neurons that display a functional and cell-autonomous phenotype which responds rapidly to manipulations that target protein synthesis. We have shown that a genetically accessible subset of layer (L) 5 cortical neurons fulfills these criteria. In acutely prepared slices of visual cortex, L5 pyramidal neurons exhibit increased neuronal spiking in *Fmr1*-KO mice that is corrected by bath application of various drugs known to normalize enhanced protein synthesis by targeting key nodes of the metabotropic glutamate receptor 5 (mGluR5) signaling cascade.

Importantly, we demonstrate that this cortical hyperexcitability is corrected within 30 minutes of protein synthesis inhibition by cycloheximide (CHX), providing the mechanistic insight that the proteins involved in maintaining cortical hyperexcitability are dynamically regulated. Furthermore, whole-cell patch-clamp recordings in the presence of synaptic blockers reveal *Fmr1*-KO L5 pyramidal cells exhibit increased input resistance and decreased rheobase current and that this cell-intrinsic hyperexcitability, like increased L5 spiking activity, is ameliorated within 30 minutes of CHX treatment. We further investigated this cell-autonomous phenotype by generating current-voltage (IV) curves that demonstrate a voltage-dependent increase in membrane resistance, suggesting deficient activity of inward-rectifying potassium channels. Current experiments are focused on identifying the proteins involved in altering this potassium channel activity, potentially uncovering novel therapeutic targets for the treatment of FXS, as well as the mechanisms for development of tolerance to treatment with mGluR5 inhibitors (See abstract by Heynen et al.).

Disclosures: M.J. Heinrich: None. **D.C. Stoppel:** None. **F. Weng:** None. **P.K. McCamphill:** None. **R.K. Senter:** None. **M.F. Bear:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allos Pharma.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.07

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH106469 (MFB)
Picower Institute Innovation Fund (MFB)
FRAXA postdoctoral fellowships (RKS and PKM)
Translational Post-doctoral Training in Neurodevelopment (TPND) Program at Boston Children's Hospital: T32MH112510 (DCS)

Title: Mglur5 negative modulators for Fragile X: resistance and persistence

Authors: *A. J. HEYNEN, **D. C. STOPPEL**, P. K. MCCAMPHILL, R. K. SENTER, M. F. BEAR;
The Picower Inst. for Learning & Memory, Cambridge, MA

Abstract: Fragile X syndrome (FXS) is caused by silencing of the human *FMR1* gene and is the leading monogenic cause of intellectual disability and autism spectrum disorder. Abundant preclinical data has indicated that negative allosteric modulators (NAMs) of metabotropic glutamate receptor 5 (mGluR5) or inhibitors of key downstream signaling nodes might be

efficacious in treating FXS in humans. Initial attempts to translate these findings in clinical trials have failed. The emergence of acquired treatment resistance ("tolerance") after chronic administration of mGluR5 NAMs is a potential factor in the lack of translational success. Here we provide evidence confirming that the mouse model of FXS (*Fmr1*-KO) displays acquired treatment resistance after chronic treatment with the mGluR5 NAM CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) in three assays that respond to acute mGluR5 inhibition: (1) audiogenic seizure susceptibility, (2) sensory cortex hyperexcitability, and (3) hippocampal protein synthesis. As compared to the ameliorative effect of a single acute dose of CTEP (I.P., 2 mg/kg) prior to experimental testing, chronic CTEP (I.P., 3 doses over 5 days) failed to reverse deficits in these three assays. Treatment resistance does not appear to be due to increased mGluR5 expression or sensitivity as it was not overcome by additional treatment with structurally distinct mGluR5 NAMs in the cortical hyperexcitability and hippocampal protein synthesis assays. Furthermore, a selective inhibitor of glycogen synthase kinase 3α (GSK3α) was unable to overcome acquired treatment resistance, but the protein synthesis inhibitor cycloheximide was still effective, positioning the mechanism of acquired treatment resistance downstream of GSK3α, but upstream of protein synthesis. The rapid emergence of tolerance to CTEP treatment begged the question of how previous studies showed an improvement in inhibitory avoidance (IA) cognitive performance after chronic treatment. We show that this observation is likely explained by timely inhibition of mGluR5 during a critical period of development as brief CTEP treatment (I.P. 2 mg/kg, 3 doses over 5 days) in juvenile mice (P28) is sufficient to provide a persistent improvement of IA behavior measured many weeks later ($\geq$ P58). These data will be important to consider when designing future fragile X clinical trials using compounds that target the mGluR5-to-protein synthesis signaling cascade.

Disclosures: A.J. Heynen: None. **D.C. Stoppel:** None. **P.K. McCamphill:** None. **R.K. Senter:** None. **M.F. Bear:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allos Pharma.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.08

Topic: A.07. Developmental Disorders

Support: NIMH Grant R01MH118827
NINDS Grant R01NS093866

Title: Fxr1 knockout alters electrophysiological properties of parvalbumin-positive interneurons in somatosensory cortex

Authors: *K. SCHEUER[1], M. SHEN[2], X. ZHAO[1], M. B. JACKSON[1];
[1]Neurosci., [2]Waisman Ctr., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Parvalbumin-positive interneurons (PVIs) are a class of fast-spiking GABAergic interneuron defined by the expression of the calcium-binding protein parvalbumin. Their short membrane time constant and capacity to fire at frequencies of more than 80 Hz enable PVIs to exert tight control over the temporal integration window of their targeted excitatory neurons. Their strong, precisely timed inhibition enables PVIs to drive and entrain gamma oscillations, rhythmic brain waves between 30-80 Hz that have been linked to numerous functions including working memory, social cognition, and perceptual binding. PVI dysfunction and gamma oscillation impairment have been implicated in psychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia which are marked by social impairment, altered sensory processing, and deficits in executive functions such as working memory. Knockout of the FMR1 autosomal homolog 1 gene (FXR1) in PVIs results in some similar deficits, including impairments in gamma oscillations, social behavior, sensory processing, and spatial learning. Because the role of FXR1 in PVI electrophysiology in the somatosensory cortex has not been investigated, we used a genetically-encoded hybrid voltage optical sensor (hVOS) to image electrical behavior in somatosensory cortex and evaluate the impact of PVI-specific FXR1 knockout (KO). We crossed an hVOS Cre reporter mouse with a parvalbumin Cre driver to generate PV::hVOS mice with hVOS probe targeted to PVIs. Electrical stimulation of somatosensory cortex in slices from these mice elicited fluorescence changes in up to 75 PVIs in a single field of view spanning multiple cortical layers. PVI::hVOS mice were bred with FXR1-floxed mice to obtain animals in which PVIs expressed the hVOS probe and also lacked FXR1. Stimulation of somatosensory cortex layer IV evoked responses that had significantly larger amplitudes in FXR1-KO PVIs than in control PVIs. In response to layer IV stimulation, FXR1-KO PVIs in layer II/III had significantly longer latencies than control PVIs. Thus, PVIs lacking FXR1 in somatosensory cortex have altered electrophysiological properties. The FXR1-related changes in PVI functional activity may be relevant to cognitive, behavioral, and psychiatric impairments reported in FXR1 KO animals.

Disclosures: K. Scheuer: None. **M. Shen:** None. **X. Zhao:** None. **M.B. Jackson:** None.

Poster

P055. Fragile X: Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P055.09

Topic: A.07. Developmental Disorders

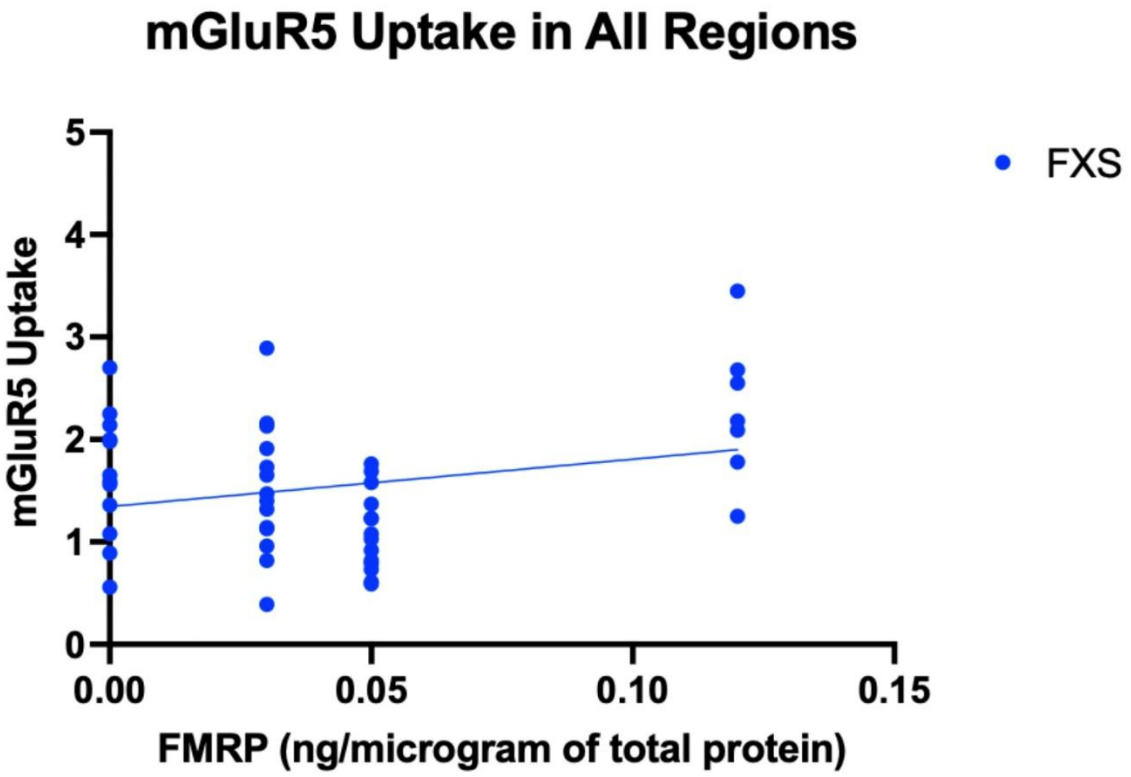
Support: Radiology Bridge/Development Funding Initiative to STimulate and Advance Research (RAD BriteStar Bridge) Award from the Johns Hopkins University School of Medicine, Baltimore, Maryland
Intellectual & Developmental Disabilities Research Center (U54 HD079123), Kennedy Krieger Institute, and Johns Hopkins Medical Institutions, Baltimore, Maryland

Title: Association of Fragile X Mental Retardation Protein (FMRP) and metabotropic glutamate receptor subtype 5 ($mGluR_5$) in fragile X syndrome (FXS)

Authors: *J. A. GOODMAN[1], S. D. MARTIN[2], D. S. RUSSELL[3], D. JENNINGS[4], O. BARRET[5], A. NANDI[2], K. J. SLIFER[6], A. K. MATHUR[2], T. W. SEDLAK[2], J. P. SIEBYL[3], E. BERRY-KRAVIS[7], D. F. WONG[8], D. B. BUDIMIROVIC[6], J. R. BRASIC[2];
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Abstract: Background: Convergent evidence indicates that deficits of Fragile X Mental Retardation Protein (FMRP) cause dysfunctional regulation pathways of metabotropic glutamate receptor subtype 5 ($mGluR_5$). This results in the neurobehavioral phenotype of fragile X syndrome (FXS); however, in several clinical trials with $mGluR_5$ negative allosteric modulators, behavioral symptoms were not improved in patients with FXS (Budimirovic, et al., 2017).
Methods: Adult men with FXS (N = 7, age 27.67 $\pm$ 4.28, range (22.3, 33.6) with the full mutation of the fragile X mental retardation 1 (*FMR1)* gene underwent quantitative measurements of FMRP by PCR/Southern Blot testing and $mGluR_5$ by positron emission tomography (PET) with 3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile ([^{18}F]FPEB).
Results: The $mGluR_5$ uptake in all brain regions (caudate nucleus, occipital cortex, parietal cortex, posterior cingulate cortex, putamen, thalamus, and temporal lobe) of men with FXS as a regression against FMFP concentration represented in the Figure suggests a trend towards significance ($p = 0.0773$).
Limitations: FXS affects a small portion of the population, and so recruiting participants presents financial and logistical constrictions. The small sample size provides a basis from which future studies may build; however, external validity is limited for the findings of this study.
Conclusions: The reductions of $mGluR_5$ in key brain regions may contribute to the neurobehavioral phenotype of FXS. Understanding this relationship between FMRP and $mGluR_5$ expression could provide the basis for the development of novel interventions for FXS.
References: Budimirovic DB, *et al*. Updated report on tools to measure outcomes of clinical trials in fragile X syndrome. J Neurodev Disord 2017, 9, 14
Martin SD, *et al*. Correlation between the Fragile X Mental Retardation Protein (FMRP) and the cerebral expression of the metabotropic glutamate receptor subtype 5 ($mGluR_5$) in fragile X

syndrome. Program No. 027-24. 2021 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2021. Online.



Disclosures: J.A. Goodman: None. **S.D. Martin:** None. **D.S. Russell:** None. **D. Jennings:** None. **O. Barret:** None. **A. Nandi:** None. **K.J. Slifer:** None. **A.K. Mathur:** None. **T.W. Sedlak:** None. **J.P. Siebyl:** None. **E. Berry-Kravis:** None. **D.F. Wong:** None. **D.B. Budimirovic:** None. **J.R. Brasic:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.01

Topic: A.07. Developmental Disorders

Title: Daddy's drinking matters: The transgenerational impact of paternal alcohol consumption on brain and behavioral development of progeny

Authors: *K. E. CONNER[1], R. F. PEREZ[2], M. NABATANZI[2], K. J. HUFFMAN[3]; [1]Neurosci., [2]Psychology, [3]Psychology/Neuroscience, Univ. of California, Riverside, Riverside, CA

Abstract: Fetal alcohol spectrum disorders (FASD) describe the wide array of long-lasting developmental abnormalities in offspring due to prenatal alcohol (ethanol [EtOH]) exposure via maternal gestational drinking. Although the teratogenic consequences of prenatal EtOH exposure are apparent, the effects of preconception paternal EtOH exposure (PatEE) are still unclear. In previous rodent models, PatEE has been shown to disturb gene expression in the brain (Finegersh and Homanics, 2014; Kim et al., 2014; Liang et al., 2014; Przybycien-Szymanska et al., 2014; Rompala et al., 2017) and liver (Chang et al., 2017, 2019) of offspring. Our previous research suggests that PatEE can induce molecular changes and abnormal behavior in the offspring in the first filial generation (F1) (Conner et al., 2020). However, it is not known whether the effects of PatEE can be passed transgenerationally. In this study, we utilized a novel mouse model of PatEE where male mice self-administered 25% EtOH for an extended period prior to conception, generating indirect exposure to the offspring (F1) through the paternal germline. PatEE (F1) males were paired with EtOH naïve females to generate offspring for the second generation (F2). The impact of paternal ethanol exposure on newborn offspring neocortical development was evaluated using neuroanatomical and molecular techniques, as well as a battery of behavioral assays in pre- and peri-pubescent mice. We observed no differences between control and PatEE (F1 and F2) mice in measures of brain weight or neocortical length. *In situ* RNA hybridization was used to investigate gene expression in the neocortex of newborn control and PatEE brains. Specifically, *Id2* and *RZRβ* expression patterns were examined; these genes play critical roles in neocortical patterning. Laminar-specific patterns of gene expression were found, where *Id2* was expressed rostromedially and *RZRβ* rostrolaterally, and expression boundaries were shifted laterally in *Id2* and medially *RZRβ* in PatEE F1 mice. Finally, two subsets of pups per filial generation were raised to ages postnatal day (P) 20 and P30 when performance was evaluated on various behavioral assays. PatEE F1 mice exhibited increased motor deficits and PatEE (F1 and F2) male mice exhibited hyperactivity compared to controls, as assessed by the Suok bar test. PatEE (F1 and F2) male mice displayed significantly reduced sensory motor integration and motor learning compared to controls, as assessed by the accelerating rotarod test. Overall, these findings suggest that ethanol exposure in males prior to conception may induce long-lasting, transgenerational changes in the brain and behavior of offspring.

Disclosures: K.E. Conner: None. **R.F. Perez:** None. **M. Nabatanzi:** None. **K.J. Huffman:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.02

Topic: A.07. Developmental Disorders

Title: Drinking while breastfeeding: Impacts on brain and behavioral development in a mouse model

Authors: *R. F. PEREZ[1], K. E. CONNER[2], M. NABATANZI[1], K. J. HUFFMAN[3]; [1]Psychology, [2]Neurosci., [3]Psychology/Neuroscience, Univ. of California, Riverside, Riverside, CA

Abstract: Maternal consumption of alcohol during pregnancy can lead to fetal alcohol spectrum disorders (FASD) in offspring. Prenatal ethanol exposure disrupts several aspects of normal brain development; however, little is known about how early exposure of ethanol contaminated breast milk may impact development. Infants exposed to ethanol via breastmilk often present with reduced body mass, verbal IQ scores, and altered sleeping patterns (Tay et al., 2017; May et al., 2016). In the U.S., approximately 36% of breastfeeding mothers consume alcohol (May et al., 2016); incidence rates are even higher in Australia (60%; Tay et al., 2017). To examine these effects, we designed a novel murine lactational ethanol exposure (LEE) model, where offspring were administered ethanol via nursing from postnatal day (P) 6 through P20. Dam and pup blood ethanol concentration (BEC) and plasma osmolality (pOsm, a measure of hydration) were measured at weaning (P20). As expected, experimental dams had significantly higher BEC levels when compared to controls, as did LEE pups at P20. Also, pOsm was unaffected by treatment. To examine sex specific impacts of our model, we analyzed data from our neuroanatomical and behavioral assays at P20 and P30 separately for males and females. Offspring body weights were significantly lower for male and female LEE offspring. Brain weights were significantly lower for male LEE offspring at P20 and P30; however, females displayed significantly lower brain weights only at P20. At P30, LEE Males displayed significantly shorter cortical lengths. Nissl staining revealed a significant decrease in size of the frontal cortex of P20 LEE mice compared to controls. However, there were no differences in prelimbic, somatosensory, auditory, and visual cortices. Additionally, spine densities were recorded for all groups via Golgi-Cox staining. Our behavioral results indicate main effects of treatment for LEE mice, with increased time spent in the open arms of the elevated plus maze and a decreased time spent immobile in the forced swim test. However, there were no significant effects observed in the accelerated rotarod, three-chambered sociability task, and Suok bar test. Our data describe potential adverse outcomes due to LEE and suggest that women refrain from drinking during breastfeeding. As there is a paucity of research examining the effects of alcohol consumption during breastfeeding, we need more studies using animal models of lactational exposure to advance our understanding and recommendations of safe maternal practices in early infancy.

Disclosures: R.F. Perez: None. **K.E. Conner:** None. **M. Nabatanzi:** None. **K.J. Huffman:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.03

Topic: A.07. Developmental Disorders

Support: NIH MH1111

Title: Next-generation phenotyping of craniofacial abnormalities in 22q11.2 deletion syndrome and in patients along the psychosis-spectrum.

Authors: *J. JEE[1], M. KRALL[1], D. MCDONALD-MCGINN[3], A. BAILEY[3], E. CHEN[3], K. CLARK[1], A. CROW[1], T. B. CROWLEY[3], K. GAISER[1], R. S. GALLAGHER[1], V. GONZENBACH[1], R. C. GUR[4], C. KOHLER[1], E. J. MCCLELLAN[1], D. MCGINN[1], P. J. MOBERG[1], K. RUPAREL[1], R. T. SHINOHARA[1], B. I. TURETSKY[1], L. K. WHITE[3], R. E. GUR[1], D. R. ROALF[2];
[2]Neuropsychiatry, [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Children's Hosp. of Philadelphia, Philadelphia, PA; [4]Univ. Pennsylvania, Philadelphia, PA

Abstract: **Background**: Minor physical anomalies (MPAs) of aberrant facial development are common in 22q11.2 deletion syndrome (22q11DS) and in psychosis-spectrum patients (PS). Given the embryological basis of facial development comparing discrete measurements of facial MPAs in individuals with and without specific genetic abnormalities may help elucidate risk for overlapping features such as psychosis. Here we use 2D photographs and the Facial Dysmorphology Novel Analysis (Face2Gene (FDNA Inc.) technology, which combines facial recognition software with clinical knowledge enabling detection of dysmorphic features and recognizable patterns of human malformations.**Methods**: 2D photographs from patients with 22q11DS (n=171), schizophrenia (SZ; n=37), clinical high risk for psychosis (CHR; n=16) and healthy individuals (HC; n=94) were collected and submitted to FDNA. The top 30 syndromes to which an individual's photograph resembled were reported along with a "gestalt" score as measurement of similarity. A series of Receiver Operating Curve (ROC) analysis, Principal Component Analysis (PCA), and follow-up t-tests were performed.**Results**: In 22q11DS, 98.2% of facial images submitted to FNDA had positive hits for 22q11DS with an average mean gestalt score (0.51). ROC analysis correctly classified 22q11DS individuals 88% of the time using only the 22q11DS gestalt score. In SZ, 65.5% of images submitted to F2G were positive hits for 22q11DS but with a lower gestalt score (0.21), but, unsurprisingly, no one syndrome could correctly identify SZ, CHR, or HC. Analysis of the whole sample found that 22q11DS could be distinguished from other groups using FNDA analysis with an AUC of 90% and psychosis-spectrum (CHR + SZ) could be distinguished from HC, but with less accuracy (AUC=68%). Average gestalt scores were significantly different between non-deleted groups: HC > SZ $t(83.67)=3.75$, $p=0.0003$ and HC > CHR $t(33.03)=3.87$, $p=0.0005$.Specifically, FNDA gestalt scores for 22q11DS explained the most variance for 22q11DS patients while gestalt scores for Fragile X Syndrome explained the most variance for psychosis-spectrum (CHR + SZ), with other syndromes contributing in both cases. **Conclusions:** These preliminary results suggest overlap in

facial dysmorphogenesis between 22q11DS and SZ patients. Future work linking measures of facial dysmorphology to clinical and neurobiological phenotypes will help identify those features most directly linked to psychosis risk. Also, to the extent that these developmental markers are evident before sub-psychotic symptoms are evident, they may allow more reliable identification of psychosis risk.

Disclosures: J. Jee: None. **M. Krall:** None. **D. McDonald-Mcginn:** None. **A. Bailey:** None. **E. Chen:** None. **K. Clark:** None. **A. Crow:** None. **T.B. Crowley:** None. **K. Gaiser:** None. **R.S. Gallagher:** None. **V. Gonzenbach:** None. **R.C. Gur:** None. **C. Kohler:** None. **E.J. McClellan:** None. **D. McGinn:** None. **P.J. Moberg:** None. **K. Ruparel:** None. **R.T. Shinohara:** None. **B.I. Turetsky:** None. **L.K. White:** None. **R.E. Gur:** None. **D.R. Roalf:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.04

Topic: A.07. Developmental Disorders

Support: R01HD098038
R01NS097808

Title: Ube3a transcript and protein levels in a novel mouse model for 15q11.2-q13.3 duplication syndrome

Authors: *J. C. GODINEZ[1], D. H. YASUI[2], J. M. LASALLE[2];
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Abstract: The duplication of human chromosomal region 15q11.2-13.3 (Dup15q syndrome) is one of the most common copy number variants in individuals with Autism Spectrum Disorder (ASD). Dup15q syndrome can result from either an intrachromosomal interstitial duplication or an extra isodicentric 15 chromosome. 15q11.2-13.3 duplications inherited from maternal, but not the paternal, chromosome result in ASD. *UBE3A*, encoding a ubiquitin E3 ligase, is the only gene within the 15q11-13 duplicated region that is expressed solely from the maternal allele in neurons. *UBE3A* is therefore thought to be the prominent molecular cause of Dup15q syndrome phenotypes. Previous mouse models studying the overexpression of *Ube3a* displayed the core behavioral traits of autism but were difficult to replicate because of unstable genetic lines. These mice were genotyped only by *Ube3a* copy number and were not bred for maintaining a single allele. Therefore, to test the hypothesis that transgenic mice with stable, extra *Ube3a* gene copies will have elevated levels of *Ube3a* transcript and protein compared to wild-type littermates, a mouse model was created by insertion of extra copies of *Ube3a* into the mouse genome using the PiggyBac-on-BAC transgenic method. We chose to focus our analysis on mice with a

chromosome 6 insert because the transgene was inserted between genes and thus less likely to affect endogenous gene expression. We genotyped transgenic and wild type mice tail DNA samples by PCR and checked for the presence of extra copies of*Ube3a*. Western blot analysis was performed, with Gapdh as the control, to quantify Ube3a protein levels in brain samples. We also isolated total RNA from brains using a QIAGEN kit and manufacturer's protocol, and performed qRT-PCR to assess the RNA transcript levels of*Ube3a*. The sample size was n=10. Sex differences were not assessed but are pending with a larger sample size. Preliminarily we found that *Ube3a* transgenic mice display higher levels of *Ube3a* transcript compared to wild-type littermates. *Ube3a* transgenic brain did trend toward higher levels of Ube3a protein compared to wild-type mice, but the results were not significant. Further tests will determine if Ube3a protein level is elevated in transgenic mice. In the future, behavioral analysis will be performed to confirm if *Ube3a* transgenic mice display phenotypes relevant to Dup15q. This novel mouse model will serve as a tool to further understand the role of UBE3A in Dup15q syndrome and to develop effective treatments.

Disclosures: J.C. Godinez: None. **D.H. Yasui:** None. **J.M. LaSalle:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.05

Topic: A.07. Developmental Disorders

Support: Angelman Syndrome Foundation

Title: Phenotyping mouse models of Angelman syndrome using multidimensional behavioral analysis

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Abstract: Angelman syndrome (AS) is a single-gene neurodevelopmental disorder caused by loss of function mutations in the maternal copy of the paternally imprinted UBE3A gene. Individuals with AS have a multifaceted behavioral phenotype consisting of deficits in motor function, epilepsy, cognitive impairments, sleep abnormalities, as well as other comorbidities. Effectively modeling this behavioral profile and measuring behavioral improvement will be crucial for the success of ongoing and future clinical trials. Foundational studies have defined an array of robust and reliable behavioral phenotypes in the AS mouse model. However, no single

behavioral test is able to fully capture the complex nature of AS - in mice, or in children. Here, we use a multidimensional analysis approach comprised of principal component analysis (PCA) and k-means clustering to quantify the combined performance across eight behavioral domains in a mouse model of AS (Ube3am-/p+). This approach can successfully predict the genotype of mice based on their behavioral profile with approximately 95% accuracy, and typically achieves >90% accuracy with an "n" of ~10 to 15 mice per genotype. We confirmed that multidimensional analysis has the necessary sensitivity to detect behavioral improvement following treatment to increase Ube3a expression in Ube3am-/p+ mice. This approach can be applied to related neurodevelopmental disorders, and may be particularly valuable for disorders where individual behavioral tests are less reliable than in AS and a combinatorial approach is required to determine a phenotype.

Disclosures: J.K. Tanas: None. **J.M. Wolter:** None. **I. Wallaard:** None. **Y. Elgersma:** None. **M.S. Sidorov:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.06

Topic: A.07. Developmental Disorders

Support: Israel Science Foundation 1454/17

Title: Enhanced activation of $Na_V1.1$ rescues synaptic properties of CA1 interneurons, but not pyramidal neurons, in a mouse model of Dravet syndrome

Authors: *Y. ALMOG[1], M. RUBINSTEIN[2];
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Abstract: Dravet syndrome is severe childhood-onset epilepsy, caused by mutations in the *SCN1A* gene, encoding for the voltage-gated sodium channel $Na_V1.1$. $Na_V1.1$ channels govern the firing and synaptic functions of inhibitory neurons, with several lines of evidence also indicating their importance to the activity of excitatory neurons. Here, we focused on synaptic evoked activity within the hippocampal CA1 microcircuit in Dravet syndrome (DS, $Scn1a^{WT/A1783V}$) and wild-type (WT) mice, assessing the contribution of $Na_V1.1$ to synaptic responses using Hm1a (50 nM), a selective $Na_V1.1$ opener. Specifically, we stimulated CA3 Schaffer collaterals while simultaneously probing the activity of excitatory CA1 pyramidal neurons (extracellular field potential or whole-cell recordings) and their postsynaptic horizontal stratum-oriens (SO) interneurons (whole-cell recordings). Stimulation of the Schaffer collaterals elicited smaller CA1 field responses in DS mice, indicating impaired synaptic activation. Interestingly, Hm1a augmented the CA1 field response in WT, but had no effect in DS.

Additionally, whole-cell recordings of CA1 pyramidal neurons demonstrated that the threshold for synaptically evoked action potential (sAP) is more depolarized in DS. Finally, while Hm1a hyperpolarized this threshold in WT neurons, it had no effect in DS. Together, these data indicate that $Na_V1.1$ channels contribute to synaptic functions and firing in WT CA1 excitatory neurons, whereas the lack of DS excitatory neurons response to Hm1a suggests that $Na_V1.1$ expression is hampered in these cells. SO interneurons presented an elevated sAP threshold, as expected. However, in contrast to the differential effect of Hm1a on CA1 pyramidal neurons, SO interneurons of WT and DS responded similarly, with reduced threshold for sAP and increased firing probability. Thus, although SO interneurons demonstrate lower excitability in DS, their $Na_V1.1$ expression level is sufficient to elicit a comparable response to Hm1a. Unexpectedly, the firing of SO interneurons per the same strength of CA3 Schaffer collaterals stimulation, was similar between WT and DS, despite the described deficits in CA1 excitatory and SO inhibitory neurons. This observation may be related to increased release probability in CA1-SO synapses, as suggested by reduced paired-pulse ratio in DS. Together, the differential effects of Hm1a on CA1 pyramidal neurons and SO interneurons indicate that DS pathophysiology is governed by complex mechanisms, with multiple neuronal and synaptic alterations occurring in both excitatory and inhibitory neurons.

Disclosures: Y. Almog: None. **M. Rubinstein:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.07

Topic: A.07. Developmental Disorders

Support: A001

Title: Identification of dysregulated proteins in the cerebral spinal fluid and plasma of a novel rat model of Angelman Syndrome

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Abstract: Angelman Syndrome (AS) is a neurodevelopmental disorder caused by mutations in the maternal allele of UBE3A. Due to neuronal specific imprinting the paternal allele is silenced

via a long non-coding RNA antisense. The loss of UBE3A in neuorns presents exciting therapeutic avenues for gene replacement via AAV or cross-correction via engineered cells or reactivation of the silent paternal allele via antisense oligonucleotides (ASO) or other genetic targeting strategies. The goal of this project was to identify, validate and test a sensitive biomarker assay to be used for any AS translational effort. Previous efforts on biomarker discovery has been performed for over a decade utilizing a variety of approaches. While clinical readouts such as EEG and sleep provide robust and reproducible phenotypes in rodent models and children with AS, the need for a molecular biomarker that can be assessed repeatedly, pre and post therapeutic intervention and non-invasively is needed. Due to the wealth of existing and published data sets we assembled a group of conserved proteins that demonstrated a significant difference between neurotypic and AS samples. These 30 candidate proteins were be assessed in multiple brain regions, cerebral spinal fluid (CSF), and plasma in a recently developed rat mpdel of AS. Additional biomarkers implicated in aging disorders but directly linked to the activity of UBE3A were also assessed. Several canididate biomarkers were identified across multiple brain regions and in the CSF of the AS rats suggestive of a potential biomarker. Future studies will evaluate the responsiveness of these measures following intrathecal administration of ASO in samples from the brain and periphery. We are also establishing a sensitive biomarker panel utilizing the Quanterix Ultra-Sensitive Biomarker Detection System. The results of this study may provide a reliable and sensitive biomarker assay to accurately predict therapeutic efficacy and could serve as a therapeutic endpoint in either translational studies using preclinical models or in clinical trials of AS.

Disclosures: D.L. Cameron: None. **L. Berg:** None. **A. Adhikari:** None. **N. Copping:** None. **J.A. Halmai:** None. **P. Deng:** None. **J.L. Silverman:** None. **D.J. Segal:** None. **K. Fink:** None.

Poster

P058. Angelman and Other Developmental Disorders: Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P058.08

Topic: D.06. Auditory & Vestibular Systems

Title: Auditory evoked oscillations are altered in UBE3A knock-out rat model of Angelman's syndrome

Authors: *I. GONZALEZ-BURGOS[1,2,3], M. BAINIER[1], P. SCHOENENBERGER[1], P. JANZ[1], M. VALENCIA[2,3], R. L. REDONDO[1];
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Abstract: Angelman's syndrome (AS) is a severe neurodevelopmental disease caused by a lack of function of a segment of chromosome 15. Most of the time it is related to a deletion or mutation of the UBE3A gene on that chromosome. The electroencephalogram (EEG) in AS is usually abnormal and may be used as a quantitative biomarker for differential diagnosis, to track progression, and as clinical outcome estimate. Here we investigate the effect of UBE3A deletion on the spontaneous activity and auditory-evoked responses by using a UBE3A knock-out rat model of AS. To do that, we explored the ongoing activity (qEEG), the auditory steady state responses (ASSR, oscillatory responses to rhythmic auditory stimuli) and the chirp-EP responses (responses related to a tone modulated in amplitude at increasing frequencies) under open-field, freely-moving conditions. Spectral analyses suggest a decrease in gamma oscillatory activity at the frontal cortex in knock-out rats when compared to wild-type. Interestingly, robust changes in the ASSR and chirp-EP were found. The 40-Hz ASSR evoked activity and intertrial coherence were increased for transgenic animals. Similarly, the chirp-EP evoked activity and intertrial coherence were increased in a region at around 60 Hz. These differences are found bilaterally in the frontal cortex but not in the parietal cortex, suggesting a region specific disruption of neuronal synchronization mechanisms in the UBE3A knock-out rats. If replicated in patients, the ASSR and chirp-EP could constitute a useful biomarker for AS, guiding pharmacological development of therapies that target aberrant neuronal function.

Disclosures: I. Gonzalez-Burgos: None. **M. Bainier:** None. **P. Schoenenberger:** None. **P. Janz:** None. **M. Valencia:** None. **R.L. Redondo:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.01

Topic: B.07. Synaptic Plasticity

Support: CIHR (Canadian Institutes of Health Research) Foundation Grant #154276

Title: Intracellular Ca2+ stores are required for synaptic tag and capture in mouse hippocampal slices

Authors: *L. A. KOEK[1,2], T. M. SANDERSON[1], J. GEORGIOU[1], G. L. COLLINGRIDGE[1,2,3]; [1]Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada; [2]Physiol., Univ. of Toronto, Toronto, ON, Canada; [3]TANZ Ctr. for Res. in Neurodegenerative Diseases, Univ. of Toronto, Toronto, ON, Canada

Abstract: Long term potentiation (LTP) is a form of synaptic plasticity vital for learning and memory and can be differentiated mechanistically into two distinct forms based on independence of (LTP1) or requirement of (LTP2) de novo protein synthesis. In addition, LTP induced by a

weak stimulus can be enhanced if it is preceded by LTP induced by a strong stimulus at neighbouring synapses due to a process known as synaptic tagging and capture (STC). The role of intracellular Ca^{2+} stores in LTP1, LTP2 and STC are not well understood.
We induced LTP using two induction protocols, a compressed theta burst protocol (cTBS) that induces LTP1, and a spaced TBS protocol (sTBS) that additionally induces LTP2. The theta bursts in these two protocols were identical (5 pulses at 100Hz repeated 5 times at 5 Hz) as were the total number of pulses (75). The only difference was the inter theta burst interval: in cTBS this was 10 seconds, in sTBS this was 10 minutes. In some experiments we followed the sTBS protocol with a weak TBS (wTBS; one round of theta burst, 25 pulses) applied to an independent pathway 30 mins later, to study STC. We recorded fEPSPs from stratum radiatum in response to stimulation of the Schaffer Collateral -Commissural pathway of 10-12 week old C57BL/6J mice in the presence of cyclopiazonic acid (CPA, 30 uM), Ryanodine (Ry, 10uM) and vehicle.
We found that sTBS induced LTP that was inhibited by both CPA and Ryanodine. In interleaved vehicle and CPA treated slices LTP, quantified 120 min after delivery of sTBS was 174.3±7.9% and 141.6±7.5% respectively, (n=5, p<0.05). In a second set of interleaved vehicle- and Ry-treated slices, LTP was 160.3±3.7 % and 136.6±2.8 % respectively (n=6, p<0.01). In contrast, LTP induced by cTBS was not affected by either compound. LTP levels were 145.1±2.7, and 147.2±3.2, respectively, in control and CPA treated slices (n=5, $p > 0.05$) and 146.3±2.8, and 137.4±2.6, respectively in control- and Ry- treated slices (n=6, $p > 0.05$). To study STC, we delivered a weak TBS after delivery of the sTBS to an independent input, as determined by lack of heterosynaptic paired-pulse facilitation. The wTBS alone induced an LTP of 103.3±7.2 %, (n=5) whereas the wTBS induced an LTP of 137.9±5.7 %, n=8 when preceded by the sTBS. However, when Ry was present during the sTBS, the level of LTP induced by the wTBS was reduced to 112.7±5.94% (n=6, p<0.05).
These results demonstrate that Ca^{2+} stores are required for the protein synthesis-dependent form of LTP (LTP2) and are also involved in STC.

Disclosures: L.A. Koek: None. **T.M. Sanderson:** None. **J. Georgiou:** None. **G.L. Collingridge:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.02

Topic: B.07. Synaptic Plasticity

Support: T32 GM007377
R01 NS06736

Title: Determining the neuronal substrates of the RhoGEF, Ephexin5

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Abstract: The activity-dependent structural plasticity of dendritic spines is a necessary component of learning and memory. Spine plasticity requires the local rearrangement of the actin cytoskeleton. Crucial for this is the activation of the Rho GTPases RhoA, Cdc42, and Rac1, whose activity control cytoskeleton dynamics, and their activators, RhoGEFs. Ephexin5 (E5) is a RhoGEF important for regulating synapse development. Previous work from the Zito lab demonstrated a role for E5 in regulating activity-dependent spine plasticity; however, the molecular pathways involved are unknown. While E5 has been shown to activate RhoA in neuronal tissue, evidence from somatic cells show E5 activates Cdc42 and Rac1. Notably, RhoA and Cdc42/Rac1 have been shown to play opposing roles in regulating dendritic spine structure, with RhoA activation inducing spine collapse and Cdc42/Rac1 activation inducing spine outgrowth and stabilization. Therefore, uncovering the full range of neuronal GTPase substrates of E5 is critical for understanding its role in spine plasticity. Crucially, the E5 homolog, Ephexin1, undergoes a phosphorylation-dependent switch in substrate preference from Cdc42/Rac1 to RhoA. It is our hypothesis that E5 undergoes a neuronal activity-dependent shift in substrate specificity from RhoA to Rac1/Cdc42, relevant to its role in regulating spine plasticity. To test this, we used 2-photon fluorescence lifetime imaging of genetically-encoded FRET biosensors for Rho GTPase activity in hippocampal CA1 neurons of E5 knockout (E5 KO) and wildtype (WT) mice. We found that, in response to glutamatergic stimulation, E5 KO neurons displayed lower Cdc42 activation compared to WT, while RhoA activation was unaltered. Intriguingly, E5 has previously been shown only to activate RhoA in neurons. Our results provide evidence that E5 activates Cdc42 during glutamatergic signaling in neurons.

Disclosures: S. Petshow: None. **K.M. Zito:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.03

Topic: B.07. Synaptic Plasticity

Support: NIH F30 MH122046
NSF IOS-1457336
NIH R01 MH109719

Title: Inhibitor-2 positively regulates PP1-neurabin synaptic activity

Authors: *K. FOLEY[1,2], T. COUCH[2], H. HOU[2], M. M. PAPASERGI-SCOTT[2], D. M. MACLEAN[2], H. XIA[1,2];
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Abstract: Protein phosphatase 1 (PP1) is a classic molecule of forgetfulness and potent regulator of post-synaptic plasticity. PP1 dephosphorylates a broad array of proteins, but requires targeting proteins to achieve substrate specificity: each function of PP1 is mediated by a PP1 holoenzyme, consisting of the PP1 catalytic subunit and a targeting protein. At the synapse, the neurabin-PP1 holoenzyme is a well-established regulator of AMPA receptor trafficking under basal and activity-dependent conditions. However, additional PP1-interacting proteins are present at the synapse, and little is known about the functional roles of the associated PP1 holoenzymes. Here we study inhibitor-2 (*PPP1R2*) and its interactions with PP1 and neurabin, as well as its functional role in synaptic transmission. Inhibitor-2 is so named by its inhibitory effect on PP1 *in vitro*, although we recently discovered that, paradoxically, inhibitor-2 positively regulates PP1 function *in vivo*. These opposing roles of inhibitor-2 on PP1 regulation may depend on the presence of, and interaction with, other PP1 targeting proteins. Using Förster resonance energy transfer (FRET)-fluorescent lifetime imaging microscopy (FLIM), we demonstrate direct interactions of inhibitor-2 with PP1 and neurabin, and key residues affecting interaction. Additionally, by performing field recordings in acute hippocampal slices, we show that knockout of inhibitor-2 replicates PP1 inhibition in CA1 synaptic function. Finally, we present a model by which inhibitor-2 could act as a positive regulator of PP1 function in the brain.

Disclosures: K. Foley: None. **T. Couch:** None. **H. Hou:** None. **M.M. Papasergi-Scott:** None. **D.M. MacLean:** None. **H. Xia:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.04

Topic: B.07. Synaptic Plasticity

Support: NIMH R01 MH120300

Title: ELAV-mediated mRNA translocation to the synapse: a candidate molecular mechanism for savings memory

Authors: *A. A. MIRISIS, A. SERRANO LUGO, J. Y. ZHAO, T. J. CAREW;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Savings memory is a phenomenon in which the molecular remnants of a “forgotten” memory are able to prime a neural system to re-express the memory following a reminder event

(which alone is insufficient to induce a *de novo* memory). In the marine mollusk *Aplysia californica*, behavioral long-term memory (LTM) for the tail-elicited siphon withdrawal reflex requires: (i) ERK activation, (ii) protein synthesis, and (iii) *de novo* transcription in the pleural sensory neurons (SNs) that mediate the reflex. Following the natural decay of LTM (forgetting), savings LTM can be established by a reminder stimulus which alone does not establish LTM in naïve animals. This process is dependent on protein synthesis but is unaffected by ERK inhibition (Menges et al., 2015). In our current work we have studied the *Aplysia* sensory neuron-motor neuron (SN-MN) system in both culture and *ex vivo* pleural-pedal ganglia. We found that, following LTM training (4 5HT pulses separated by 15 min), the RNA-binding protein ApELAV1 translocates in a microtubule-dependent manner from the SN nucleus into synaptic/dendritic processes. We have further determined that the mRNA encoding the transcription factor ApC/EBP, an ELAV-target RNA (Mirisis et al., 2021), is localized to distal SN compartments (including SN-MN synapses) after LTM training. Translation of this mRNA by subsequent savings training could provide a molecular mechanism for the re-establishment of LTM following its natural decay. In support of this hypothesis, we have found that savings training induces expression of ApC/EBP protein in previously trained, but not in naïve animals. The mechanisms underlying this expression of savings memory are distinct from the mechanisms underlying its expression in initial LTM training. Our findings thus identify a set of novel molecular mechanisms which can facilitate relearning, and interestingly, are distinct from those mechanisms that are required to establish original LTM.

Disclosures: A.A. Mirisis: None. **A. Serrano Lugo:** None. **T.J. Carew:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.05

Topic: B.07. Synaptic Plasticity

Support: DGAPA-PAPIIT 208518
DGAPA-PAPIIT 215719
CONACyT 308538

Title: Progression of granule cell dispersion occurs along with in vivo LTP reinstatement and contextual fear memory recovery

Authors: *K. HERNÁNDEZ MERCADO[1], A. MARTÍNEZ MORENO[2], L. RODRÍGUEZ DURAN[2], M. L. ESCOBAR[2], A. ZEPEDA[1];
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Abstract: The dentate gyrus (DG) is a structure through which multiple sensory information coming from the perforant pathway (PP) enters the hippocampus. The adequate integration of such information in the DG is critically important for the execution of hippocampal-dependent cognitive functions. Granular cell dispersion (GCD) is a pathological widening of the DG granular cell layer frequently observed in humans with mesial temporal lobe epilepsy (MTLE). Until now, it is unclear whether synaptic plasticity and hippocampal-mediated cognitive behaviors are affected by the presence and severity of GCD in MTLE. To evaluate the latter, we used the intrahippocampal injection of kainic acid (KA) mouse model of MTLE and analyzed in the temporal development of GCD: 1) the establishment of in vivo long-term potentiation (LTP) in the PP-DG pathway, and 2) the performance in learning and memory through contextual fear conditioning. Our results show that the gradual increase in GCD severity along time was accompanied by a synaptic reorganization and functional cognitive recovery. Impairment in LTP induction along deficits in learning and memory were observed as GCD subtly developed at 10 days-post-lesion (dpl). However, at 30 dpl a severe increase in GCD occurred which was surprisingly accompanied by the restoration of LTP induction and memory recovery. These findings support the hypothesis that GCD emerges as a local homeostatic change that maintains the physiological function of the DG network.

Disclosures: K. Hernández Mercado: None. **A. Martínez Moreno:** None. **L. Rodríguez Duran:** None. **M.L. Escobar:** None. **A. Zepeda:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.06

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 120300-02
NIH Grant 126613-01

Title: Proteolytic processing of growth factor TGFβ provides a mechanism for precise temporal orchestration in long term memory formation

Authors: *P. MIRANDA, N. KUKUSHKIN, A. A. MIRISIS, M. SCHREIBMAN, T. J. CAREW;
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Abstract: While typically viewed as hormone-like molecules operating with little temporal resolution, growth factors can also play key roles in orchestrating the timing of molecular events required to form a long-term memory (LTM). In a two-trial training paradigm that induces LTM for sensitization in the marine mollusk *Aplysia californica*, growth factor TGFβ is critically

required during Trial 2 but not Trial 1 (Kopec et al, 2015). This finding suggests a temporally specific inter-trial regulation of TGFβ's signaling cascade, either upstream or downstream of TGFβ release. Since TGFβ is necessary and sufficient to promote LTM, we hypothesized that it contributes to the inter-trial interactions that determine the temporally restricted requirement of repeated trial learning (Miranda et al 2021). However, the precise mechanisms that determine the differential requirement for TGFβ during two trial training remain unknown.To examine whether interactions between trials alter the magnitude of TGFβ signaling, we have developed a bioassay based on the nuclear translocation of Smad, a downstream target of the TGFβ cascade. Cultured *Aplysia* sensory neurons were treated with either one or two pulses of 5HT to mimic two-trial sensitization training in the intact animal. We found that the rate of Smad nuclear translocation is indeed significantly increased after Trial 2 compared to Trail 1, suggesting differential activation of the TGFβ cascade during two trial training. To investigate the mechanism underlying this effect, we monitored Smad translocation in the presence of BMP-1, a protease known to convert secreted TGFβ from a latent to an active state. While BMP-1 alone had no effect on Smad translocation, when combined with a single pulse of 5HT, it significantly enhanced Smad's redistribution. Finally, in a gain of function experiment, we found that a single pulse of 5HT, when paired with BMP-1, induces long-term synaptic facilitation (LTF) in cultured sensory-motor neurons, whereas a single 5HT pulse alone does not induce LTF. These data support a model in which the interaction between the two trials involves both temporally segregated stages of TGFβ release and its proteolytic activation, the combination of which is required for the precise temporal processing during LTM formation.

Disclosures: P. Miranda: None. **N. Kukushkin:** None. **A.A. Mirisis:** None. **M. Schreibman:** None. **T.J. Carew:** None.

Poster

P108. LTP: Kinases and Intracellular Signaling I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P108.07

Topic: B.07. Synaptic Plasticity

Support: NIH grant 1R01NS092705
NIH grant 1R01NS107453
NIH grant T32NS007453-16
Division of Neurology, CCHMC
Cincinnati Children's Research Foundation
University of Cincinnati Neuroscience Graduate Program

Title: Loss of MicroRNA-324-5p alters cytoskeletal protein expression, modifies dendritic spine morphology, reduces dendritic spine density, and impairs long-term potentiation in the hippocampus

Authors: *E. V. PARKINS[1,3], D. H. BRAGER[7], J. K. RYMER[3], J. M. BURWINKEL[3], D. ROJAS[3], Y.-C. HU[4,5], D. TIWARI[3,6], C. GROSS[3,2];
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Abstract: MicroRNAs are small noncoding RNAs that post-transcriptionally regulate gene expression. Their ability to rapidly alter gene expression and target multiple pathways makes them interesting candidates in the study of synapses, which are both dynamic and highly regulated. Here, we demonstrate that miRNA-324-5p regulates dendritic spines, small protrusions along the dendrite that comprise the majority of excitatory synapses in the brain. Using intracerebroventricularly (ICV) administered antagomir (inhibitor) of miR-324-5p in Thy1-eGFPhemi mice, we demonstrate that acute loss of miR-324-5p leads to a reduction in dendritic spine density and altered spine morphology in the hippocampus, with fewer thin and more stubby spines. To investigate the effect of chronic loss of miR-324-5p, we generated a CRISPR-Cas9-mediated Mir324 knockout (miR324 KO) mouse model and completed Golgi-cox staining to assess spine density. We also crossbred miR-324 KO mice with Thy1- $eGFP^{hemi}$ mice to assess spine density and morphology. Interestingly, we found reduced spine density and the same changes in morphology with both acute and chronic miR-324-5p loss. RNA-sequencing identified altered biological pathways in the miR-324 KO hippocampus that may contribute to the mechanism of miR-324-5p-mediated spine regulation, including cytoskeletal organization. Western-blot and quantitative Real-Time PCR also revealed changes in protein and mRNA levels for potassium channels, cytoskeletal components, and synaptic markers, including MAP2, a cytoskeletal protein that is essential for long-term potentiation (LTP). Intriguingly, slice electrophysiology revealed that LTP is severely impaired in miR-324 KO mice, while baseline excitatory activity remained unchanged. Altogether, our findings suggest that miR-324-5p is an important regulator of dendritic spines, and that its loss has brain functional consequences. Future studies will explore the mechanism by which miR-324-5p regulates dendritic spines and the role it plays in LTP, for example its role in dendritic spine dynamics during potentiation. We will also explore miR-324-5p antagomir's potential to reduce aberrant dendritic spine density in mouse models of autism. Both male and female mice were used in these studies, and no sex differences were detected. Experimenters and analyzers were blind to conditions, and studies were fully powered (power≥0.8) or indicated as preliminary.

Disclosures: E.V. Parkins: None. **D.H. Brager:** None. **J.K. Rymer:** None. **J.M. Burwinkel:** None. **D. Rojas:** None. **Y. Hu:** None. **D. Tiwari:** None. **C. Gross:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.G. is co-Inventor on US patent 9,932,585 B2..

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.01

Topic: B.11. Glial Mechanisms

Support: NIH Grant F32MH124298
URMC SPIN Grant

Title: Frontal cortical microglia respond to dopaminergic signals in the adolescent mesofrontal circuit

Authors: *R. D. STOWELL, K. H. WANG;
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Abstract: Psychiatric disorders such as schizophrenia are recognized as developmental disorders with complex disruptions in neuronal circuitry. Research in both psychiatric patients and animal models indicate that mesofrontal dopaminergic circuit abnormalities are involved in cognitive deficits. Furthermore, mesofrontal dopaminergic projections have a unique capacity for activity-dependent plasticity not found in adult animals. However, the mechanisms behind this adolescent plasticity process remain unclear. Microglia, the resident immune cell of the central nervous system, are integral participants in numerous developmental synaptic pruning and synaptic plasticity processes. Recent evidence has shown that microglia also respond *in vivo* to specific neurotransmitter signals including norepinephrine. Here utilizing a combination of transgenic, chemogenetic, and viral transduction strategies, we investigated if microglia are key participants in adolescent plasticity of mesofrontal dopaminergic circuit in mice. Utilizing chronic *in vivo* two-photon microscopy we were able to track fluorescently labelled microglia and dopaminergic axons in the M2 frontal cortical region pre- and post- stimulation of ventral tegmental area (VTA) dopaminergic neurons. We found that microglia respond to increased dopaminergic release from both chemogenetic stimulation of the VTA and wheel-running driven VTA activity. The morphology of microglial arbors changed after both chemogenetic and behavior stimulation. Microglial processes also made putative contacts with axonal elements of mesofrontal dopaminergic projections. Pharmacological manipulations further suggest that the microglial responses may be partially mediated by D2-type dopaminergic receptor signaling. Together, our results reveal an interaction between dopaminergic input and microglial activity in the adolescent frontal cortex. Further experiments will address if these microglial responses to dopaminergic signaling are essential for the adolescent development and plasticity of dopaminergic circuits in the frontal cortex. An improved understanding of these mechanisms will facilitate the identification of therapeutic targets for neurodevelopmental disorders.

Disclosures: R.D. Stowell: None. **K.H. Wang:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.02

Topic: B.11. Glial Mechanisms

Support: NIH/NHLBI (HL135635)
VA Merit Award (BX004294)

Title: Neuronal ADAM17 mediated shedding of CX3CL1 contributes to microglial activation and inflammation

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Abstract: Microglia is the principal immune cell in the brain and, once activated, releases cytokines contributing to neuroinflammation and sympathetic over-activation. Proinflammatory cytokines-mediated activation of microglia in the hypothalamus contributes to neurogenic hypertension.
Mouse Neuro-2a (N2A) cells were treated with DPC333 (ADAM17 inhibitor, 100 nM) for 24 h, followed by Ang-II (100 nM) stimulation. After 4 h, cells and media were harvested. The N2A conditioned media was added to human (HMC3) (ATCC® CRL-3304™) or Primary Microglia cells and incubated for 24 h. HMC3 cells and media were then harvested for estimation of ACE2 enzyme activity, protein expression of ACE2 and Iba1 and estimation of cytokines such TNFα, IL-6 and CX3CL1. A subset of cells were also used for IHC. CX3CL1 was identified as one of the inflammatory cytokines released from neurons after Ang-II stimulation, contributing to microglia activation and inflammation. Ang-II stimulation induced a 4-fold increase in CX3CL1 levels in N2A cells media (n=3: one-way ANOVA, $P<0.001$), implying ADAM17-mediated shedding of CX3CL1. When the Ang-II-treated N2A cells conditioned media was added to microglial cells, it resulted in a 4-fold increase in ACE2 activity and a 2-fold increase in Iba1 expression compared to untreated microglial cells (n=3: one-way ANOVA, $P<0.01$, $P<0.001$). However, microglial cells treated with N2A conditioned media exposed to DPC33 alone and DPC33+Ang-II, exhibited significantly attenuated ACE2 shedding and Iba1 levels. These data suggest that Ang-II-induced activation of neuronal ADAM17 and CX3CL1 shedding contribute to ACE2 shedding and the increase in Iba1 levels in microglia cells, respectively, promoting microglia activation and inflammation. In addition, histology data show that untreated microglial cells maintain a triangular shape with few branches, whereas cells exposed to Ang-II-treated N2A conditioned media form a network with many ramifications, which is the morphological presentation of activated microglia.
Together, we show that neuronal ADAM17-mediated shedding of CX3CL1 contributes to ACE2

shedding and activation of microglia. Therefore, inhibition of neuronal ADAM17 mediated shedding of CX3CL1 may prevent microglia activation and inflammation.

Disclosures: N. Lakkappa: None. **E.D. Lazartigues:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.03

Topic: B.11. Glial Mechanisms

Support: Presupuesto interno Facultad de Medicina, UNAM
Asociacion para evitar la ceguera en México APEC
Bioterio Instituto de Biología Celular

Title: IdentificaciÓN de la isoforma vegfc y su receptor transmembrana vegfr2 en células gliales de müller

Authors: *Z. CARRANZA JIMENEZ[1], L. OCHOA DE LA PAZ[2];
[1]Univ. Nacional Autónoma De México, Coyoacan, Mexico; [2]Univ. Nacional Autónoma de México, COYOACAN, Mexico

Abstract: Identification of the VEGF-C isoform and its transmembrane RECEPTOR VEGFR2 in Müller's glial cells. Authors * Carranza Jiménez Zoe Pamela[1,2], Ochoa-de la Paz Lenin David[1,2]. Laboratory of Molecular and Cellular Neurobiology of Glia, Department of Biochemistry, Faculty of Medicine, UNAM, Mexico City, Mexico[1.] Research Department, Association to Prevent Blindness in Mexico IAP Hospital "Dr. Luis Sánchez Bulnes" APEC, Mexico City, Mexico[2]. Summary Müller's glial cells are the main macroglia of the retina. They extend from the layer of ganglion cells to the inner segments of the photoreceptors, facilitating physical contact with many types of retinal cells. So it plays an important role in various physiological processes of the retina. In pathological conditions such as diabetic retinopathy induce the synthesis and release of inflammatory and angiogenic factors from Müller's glial cells that contribute to the processes of neurovascular dysfunction in these pathologies. Several observations in recent years indicate that Müller's glial cells are an important source of VEGFA under pathological conditions. The VEGF family consists of five members, structurally related to three different tyrosine kinase receptors (VEGFR1, VEGFR2 and VEGFR3). VEGFC is structurally related to VEGFA, induces lymphangiogenesis, increases vascular permeability and the release of inflammatory factors (IL). This VEGF isoform is attached to VEGFR2 and VEGFR3. Considering that Müller's glial cells are in contact with the blood retina, the dysfunction of Müller's glial cells can contribute to the angiogenic processes of the retina and also to the release of inflammatory factors. In this work, we characterize the presence of the

VEGF-C isoform and VEGFR-2 in Müller's glial cell culture. Müller's glia cell culture was obtained from retinas of dissociated postnatal mice. For control, we use ARPE-19 cell lines. The results indicate the presence of VEGF-C transcription in Müller's glial cells and the ARPE 19 cell line, however, transcription is more important than the ARPE-19 cell line. This result was confirmed by immunomarking assays, in which the VEGF-C isoform is expressed in the Müller glial cell soma. VEGFR-2 showed an expression of this receptor along with Müller's glial cells. So far, these results indicate the presence of VEGF-C and its receptor in Müller's glial cells, suggesting that these glial cells are the main source of VEGF-C. On the other hand, the presence of VEGFR-2 could imply a mechanism of self-regulation in the process of release of VEGF-C under pathological conditions.

Disclosures: Z. Carranza Jimenez: None. **L. Ochoa de la Paz:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.04

Topic: B.11. Glial Mechanisms

Support: ALIANZA UCMX / Innova UNAM Grant 013-2020.
UNAM-DGAPA- PAPIIT-GI200121
CONACYT-CB-238744
CONACYT-CB-283279
UNAM-DGAPA-POSDOC

Title: The expression of Angiotensin-Converting Enzyme type 2 (ACE2) in rat brain: a circuit analysis and possible implications for COVID-19 neurological manifestations

Authors: V. S. HERNANDEZ[1], ***M. A. ZETTER**[1], I. HERNÁNDEZ-ARAIZA[3], E. CAMPOS[2], L. ZHANG[1];
[1]Dept. of Physiology, Fac. of Med., [2]Physiol., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [3]Sch. of Med., Univ. of Maryland, Baltimore, MD

Abstract: The angiotensin-converting enzyme type 2 (ACE2) has gained attention as it acts as the receptor for the SARS-CoV-2, and because its dysregulation is associated with COVID-19 pathophysiology. The frequent neurological manifestations during and after the acute infection have raised the interest in its expression at nervous tissue and despite it has been detected at the single-cell level, its anatomical distribution at the level of neuronal circuits has not been yet addressed. We examined the neuronal and glial expression of ACE2 and its distribution in the rat brain. ACE2 was detected in astrocytes and its endfeet processes, at pericytes, and endothelial cells, key components of the blood-brain barrier, suggesting its possible participation in the viral

entrance to nervous tissue. ACE2 was found ubiquitously present in brain vasculature, with the highest density of capillaries expressing the enzyme in the olfactory bulb; the hypothalamic paraventricular, supraoptic, and mammillary nuclei; the midbrain substantia nigra and ventral tegmental area; and hindbrain pontine nucleus, pre-Bötzinger complex, and the nucleus of *tractus solitarius*. Furthermore, we found discrete neuronal groups immunopositive for ACE2 in brainstem respiratory rhythm generating centers, including the pontine nucleus, the parafascicular/retrotrapezoid nucleus, parabrachial nucleus, Bötzinger, and pre-Bötzinger complexes and in the nucleus of *tractus solitarius*; in the arousal-related pontine reticular nucleus and *gigantocellular* reticular nuclei. In brainstem aminergic nuclei, mainly related to reward, as the substantia nigra, ventral tegmental area, dorsal raphe, and locus coeruleus, ACE2 was expressed in tyrosine-hydroxylase positive neurons. In the epithalamic habenula, thalamic paraventricular and supramammillary nuclei positive neurons were found. At the hippocampus, pyramidal neurons were positive in all CA regions, as well as abundant ACE2 positive astrocytes. The identification of these ACE2-expressing hubs in the rat brain within well-established functional circuits facilitates the prediction of possible neurological manifestations derived from the dysregulation of brain ACE2 in humans and could serve as a reference for future translational investigation.

Disclosures: V.S. Hernandez: None. **M.A. Zetter:** None. **I. Hernández-Araiza:** None. **E. Campos:** None. **L. Zhang:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.05

Topic: B.11. Glial Mechanisms

Title: Long-term effects of adolescent THC exposure: role of prenatal environment

Authors: *K. MURLANOVA, Y. JOUROUKHIN, M. PLETNIKOV;
State Univ. of New York at Buffalo, Buffalo, NY

Abstract: The two-hit hypothesis of adult psychopathology predicts that a combination of prenatal and postnatal environmental insults increases risks for major psychiatric disorders. We generated a mouse model of environment x environment (E x E) interaction based on maternal immune activation (MIA) as a “first hit” and adolescent Δ^9-tetrahydrocannabinol (THC) exposure as a “second hit”. Both environmental factors were associated with psychiatric conditions, hence we hypothesized that MIA would interact with adolescent THC, to synergistically produce neurobehavioral abnormalities in adulthood. MIA was induced by administration of a viral mimetic, polyriboinosinic-polyribocytidilic acid [Poly (I:C), 5 mg/kg; IP] to gestating dams (CD1 mice, GD 12.5) and was validated by detecting increased levels of

IFN-γ, IL-1β, IL-6 and TNF- α in maternal serum. Male and female offspring received THC (8 mg/kg; SC) or vehicle during adolescence (PND 30-51) and were evaluated in a series of behavioral tests as adults (PND 72-90). We found that E x E interaction increased exploratory behaviors in the Hole Board Test (increased head dipping), the Open Field (increased rearing), Elevated Plus Maze (increased open space exploration) and elevated sensitivity to amphetamine (2 mg/kg, IP) in female but not male mice. Our data indicate that E x E interaction synergistically produces neurobehavioral alterations (hyperdopaminergic state) in adult mice in a sex-dependent manner. On-going studies aim at identifying the cell type-specific molecular mechanisms underlying E x E interaction in this model.

Disclosures: K. Murlanova: None. **Y. Jouroukhin:** None. **M. Pletnikov:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.06

Topic: B.11. Glial Mechanisms

Support: NIH/NIA R01AG060195
DoD W81XWH2010368
DoD W81XWH2010371
The Orchard Foundation
Harold and Ronna Cooper Family
The Consolidated Anti-Aging Foundation

Title: Parkinson's disease relevant stressors induce neutral lipid changes in primary rodent astrocytes and microglia

Authors: *R. THOMAS, P. ROWICKA, O. ISACSON, P. J. HALLETT;
McLean Hospital/ Harvard Med. Sch., Belmont, MA

Abstract: Dysregulation of lipid metabolism has been identified to be a central pathway perturbed in Parkinson’s disease (PD) and related dementias. Elevated glycosphingolipid levels, reduction in the activity of the lysosomal hydrolase, glucocerebrosidase, and cell type-specific changes in the distribution of neutral lipids in neurons and glia are observed in PD mouse models and human PD cells and tissues (Rocha EM et al., 2015; Huebecker M et al., 2019; Thomas R et al., 2021; Brekk O et al., 2020). Furthermore, high resolution microscopic analysis of Lewy bodies from post-mortem human PD brains shows that they are composed of abundant lipid membranous structures along with other dystrophic organelles such as mitochondria and lysosomes (Shahmoradian S et al., 2019). Taken together, these studies suggest a potential pathological role for lipid dyshomeostasis in the etiology of neurodegenerative disorders. To

investigate the effect of PD relevant cellular insults on intracellular neutral lipid levels, primary rodent-derived astrocytes and microglia were treated with varying concentrations of mitochondrial depolarizing agents valinomycin and rotenone, and toll-like receptor (TLR) -3 and -4-dependent inducers of inflammation, Poly(I:C) and LPS. The cell cultures were microscopically analyzed using the neutral lipid probe BODIPY. Elevated BODIPY signals were observed in both astrocytes and microglial cells upon rotenone and valinomycin treatments. Murine macrophage cells co-treated with rotenone and the acyl CoA synthetase inhibitor TriacsinC showed reduced BODIPY signal intensity from lipid droplets (LDs), confirming that LD induction in response to rotenone is the result of *de novo* synthesis of triacylglycerols. BODIPY induced changes were also seen in LD analysis of astrocytes and microglia following exposure to Poly(I:C) and LPS. These data show that cellular stressors relevant to PD induce neutral lipids in astrocytes and microglial cells. Current work is investigating how cellular stressors and lipid changes are altered in neuron-glia co-cultures and the mechanisms that lead to these changes, which will help to identify cell-cell interactions between neurons and glia in PD and related disorders.

Disclosures: R. Thomas: None. **P. Rowicka:** None. **O. Isacson:** None. **P.J. Hallett:** None.

Poster

P171. Glia-Neuron Interactions in Injury and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P171.07

Topic: B.11. Glial Mechanisms

Title: Roles of programmed cell death protein-1 (PD-1) in spinal cord microglia activation and mechanical pain in mice of different sexes

Authors: *S. RAHMAN[1], O. CHEN[2], Z. WANG[1], R.-R. JI[2];
[1]Ctr. for Translational Pain Medicine, Dept. of Anesthesiol., [2]Ctr. for Translational Pain Medicine, Dept. of Anesthesiology, Dept. of Cell Biol., Duke Univ., Durham, NC

Abstract: The blockade of programmed cell death protein-1 (PD-1) with antibodies such as nivolumab has been proven to be an effective treatment for various cancers by immunotherapy. Nivolumab is capable of reaching the brain in patients with a compromised nervous system, and PD-1 could modulate pain and cognitive function in these cases. However, the relationship between the nervous and immune systems and the effect of PD-1 inhibition on the nervous system are not yet fully understood. We recently reported that PD-1 activation in primary sensory neurons inhibits neuronal excitability and pain[1]. PD-1 also mediates opioid receptor signaling in nociceptive neurons, leading to altered opioid analgesia in rodents and nonhuman primates[2]. To explore the role of PD-1 in microglial signaling, we generated Pdcd1-Cre-reporter mice. Nerve injury induced profound PD-1 expression in a subset of microglial cells in the spinal

cord of Pdcd1-reporter mice. We also analyzed the size and number of microglia in mouse spinal cords sections before and after treatment with the PD-1 antibody nivolumab using confocal microscopy imaging. IBA1 immunohistochemistry staining was performed to label microglia in the spinal cord dorsal horn. We found that the intrathecal (spinal) injection of nivolumab (5 μg) not only induced mechanical allodynia, but also caused a robust microglial reaction with increased cell numbers and enlarged cell bodies. Furthermore, nivolumab-induced mechanical allodynia was blocked by the intrathecal injection of minocycline, a microglial activation inhibitor, in male, but not female, mice. Our findings suggest that PD-1 serves as a negative regulator of microglial activation and neuropathic pain. Furthermore, this data supports sex dimorphism in the microglial regulation of neuropathic pain. These results align with previous studies, which have shown that PD-1 inhibition induces hyperalgesia and the blockage of antinociception, and future studies can further elucidate this relationship to improve and expand on current immunotherapy strategies for chronic pain and cancer patients.

References

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2. Wang Z, Jiang C, He Q, Matsuda M, Han Q, Wang K, Bang S, Ding H, Ko MC, Ji RR. Anti-PD-1 treatment impairs opioid antinociception in rodents and nonhuman primates. Sci Transl Med 2020; 12(531).

Disclosures: S. Rahman: None. **O. Chen:** None. **Z. Wang:** None. **R. Ji:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.01

Topic:

Title: Combining single cell chromatin landscape and gene expression with FFPE spatial transcriptomic approaches to characterize Alzheimer’s disease (AD) progression in TgCRND8 APP transgenic mice

Authors: *L. J. ALVARADO[1], S. WILLIAMS[1], V. GIANGARRA[1], A. PULEO[1], K. MILLER[1], Y. LEVITES[2], K. DILLON[2], S. PROKOP[2], S. TAYLOR[1];
[1]10x Genomics, Pleasanton, CA; [2]Univ. of Florida, Gainesville, FL

Abstract: Alzheimer’s Disease (AD) currently impacts 6 million Americans who are living with the disease and 1 in 3 seniors die with a diagnosis of AD or dementia. Given the epidemiological state and impact of AD, a better understanding of the biology of the disease is needed to develop effective preventative and curative therapies. Here, we demonstrate the benefits of combining both single cell and spatial information in TgCRND8 APP transgenic mice to further understand

the progression of AD. Using the Chromium Single Cell Multiome ATAC + Gene Expression and Visium Spatial Gene Expression with FFPE assays, the open chromatin landscape and gene expression profiles of Tg APP-overexpressing and wild-type mice brains from 2 to 20 months old mice were evaluated. In addition, Aβ production and Aβ-associated neuroinflammation across several anatomical regions of the brain were analyzed and correlated with regulatory programs identified based on multiomic data. Results showed multi-cellular gene co-expression networks at both the single nucleus and spatial levels as well as distinct open chromatin sites during the course of evolution of AD pathology. These findings were noteworthy in brain regions containing Aβ plaques accompanied by a neuro-immune response from distinct cell populations as a function of disease progression. Together, the data showcases the utility of combining open chromatin and gene expression analysis at the single cell level with spatial transcriptomics of FFPE tissues to fully characterize cellular networks and responses in the time-sensitive context of AD.

Disclosures: L.J. Alvarado: A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **S. Williams:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **V. Giangarra:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **A. Puleo:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **K. Miller:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **Y. Levites:** None. **K. Dillon:** None. **S. Prokop:** None. **S. Taylor:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.02

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

Title: Spatially resolved transcriptomics in the APPSWE [Tg2576] mouse model of Alzheimer's disease

Authors: C. R. UYTINGCO, A. D. HARTNETT, J. CHEW, N. WEISENFELD, S. R. WILLIAMS, S. B. ZIRALDO, F. MESCHI, *K. R. MILLER, Y. YIN;
10X Genomics, Pleasanton, CA

Abstract: Identifying individual cells and their genetic makeup is critical for understanding their roles in how the central nervous system (CNS) functions in both healthy and diseased states. Here we demonstrate characterization of spatial transcriptomic patterns in the APPSWE [Tg2576] mouse model of familial Alzheimer's Disease (Taconic) using the Visium Spatial Gene Expression Solution. The assay involves placing serial coronally sectioned fresh-frozen tissue samples onto Visium Spatial Gene Expression slides. The slides consist of ~5,000 spot arrays with uniquely barcoded probes enabling the capture of native mRNA. From the captured molecules, cDNA were generated and libraries constructed and sequenced on an Illumina NovaSeq. Using the Space Ranger analysis pipeline and the Loupe Browser desktop software, the RNA-seq data were merged with H&E and immunostained images of the same tissue to align reads and to perform clustering and gene expression analysis. With the generated data, we demonstrated an improved version of the spotted oligo array technology that increases tissue coverage and spatial resolution with reduced spot size, and increased spot number and packing density. We captured spatial patterns of gene expression and mapped the information back to H&E and immunostained images with regional annotations. Correlation analysis demonstrated differential spatial gene expression between the control and APPSWE [Tg2576] mouse model. Together, this confluence of imaging and sequencing is a valuable tool for understanding the relationships between CNS cell types in healthy and diseased samples with an unbiased anatomical and gene expression-driven method.

Disclosures: C.R. Uytingco: A. Employment/Salary (full or part-time):; 10x Genomics. **A.D. Hartnett:** A. Employment/Salary (full or part-time):; 10x Genomics. **J. Chew:** A. Employment/Salary (full or part-time):; 10x Genomics. **N. Weisenfeld:** A. Employment/Salary (full or part-time):; 10x Genomics. **S.R. Williams:** A. Employment/Salary (full or part-time):; 10x Genomics. **S.B. Ziraldo:** A. Employment/Salary (full or part-time):; 10x Genomics. **F. Meschi:** A. Employment/Salary (full or part-time):; 10x Genomics. **K.R. Miller:** A. Employment/Salary (full or part-time):; 10x Genomics. **Y. Yin:** A. Employment/Salary (full or part-time):; 10x Genomics.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.03

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

Support: AG017617

AG014449
AG043375
AG072599

Title: Profiling gene expression in single cell populations within the frontal cortex of postmortem human tissue in Alzheimer's disease and control subjects

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that accounts for the majority of dementia cases affecting an estimated 6.2 million Americans. Currently, there is no single test for AD and diagnosis can only be confirmed by postmortem neuropathological assessment. None of the current pharmacological treatments available significantly slow or stop the damage or destruction of neurons that cause AD, providing only symptomatic relief without treating the source of disease onset or progression. While the hallmark of AD pathology is defined as the presence of amyloid-beta plaques and neurofibrillary tangles, it is still not well understood how they contribute to neurodegeneration. It is critical we understand mechanisms underlying AD to develop early biomarkers and therapies. Recent 'omics platforms (e.g., transcriptomics, proteomics, metabolomics) technical advancements enable assessment of numerous genes, proteins, metabolites, and impacted pathways through bioinformatic inquiry, which may allow a better unbiased assessment of disease mechanism(s) and potential therapeutic interventions. Currently, AD pathophysiology at the single population and circuit levels may be best dissected by examining transcriptomic analyses, since rigorous proteomic and metabolomic assessments are technically challenging using postmortem brain tissues at the present time. Accordingly, RNA sequencing (RNA-seq) provides a profile of expressed genes as well as noncoding RNAs (ncRNAs) within a given population. However, a limitation is that bulk-tissue resolution masks complex alterations occurring across cell types. Therefore, we are performing RNA-seq on single-cell populations isolated using laser capture microdissection (LCM). Using LCM, we isolate Nissl-stained pyramidal neurons from Layer III or Layer V within the frontal cortex (Brodmann area 9) utilizing postmortem human brain tissue obtained from clinically and pathologically characterized AD and age-matched nondemented control patients. RNA species from these neurons are isolated, converted to cDNA library preparations, and then analyzed via RNA-seq with subsequent bioinformatic pathway analyses (e.g., IPA, KEGG, and GO) to identify changes between control and AD patients. By studying the molecular fingerprints of selected cells known to be vulnerable to AD, we can gain insight into mechanistic changes that will enable unbiased pathway analyses through bioinformatics approaches that we expect to help explain circuit degeneration and inform novel therapeutic strategies.

Disclosures: A. Labuza: None. **M.J. Alldred:** None. **A. Saltzman:** None. **S.D. Ginsberg:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.04

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

Support: AG014449
AG017617
AG043375
AG072599

Title: Deep and superficial CA1 pyramidal neurons show distinct expression profiles via single population RNA-seq in a mouse model of Down syndrome (DS) and Alzheimer's disease (AD)

Authors: *M. J. ALLDRED[1,3], S. C. PENIKALAPATI[1], S. LEE[2,4], A. HEGUY[5], S. D. GINSBERG[1,3,6,7];
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Abstract: Individuals with Down syndrome (DS) have intellectual disability and develop hallmark Alzheimer’s disease (AD) pathology during midlife. Several circuits underlying memory and executive function in the DS and AD brain are vulnerable to neurodegeneration, notably the interconnected basal forebrain cholinergic system and the hippocampus. Murine studies demonstrate CA1 pyramidal neurons have distinct sublayers along the radial axis, namely deep and superficial neurons, that also differ along the transverse axis with regard to innervation and function. The mediolateral innervation pattern of these neuronal subpopulations is postulated to impact both spatial and non-spatial learning and memory. We performed laser capture microdissection to isolate medial deep and superficial CA1 pyramidal neuron populations in concert with single population RNA-seq and subsequent bioinformatic inquiry to determine if transcriptomic differences exist within these CA1 pyramidal neuron subpopulations. Gene expression profiles were accrued from the Ts2 trisomic mouse model of DS and AD, which presents phenotypic and pathological features similar to the human condition including age-related synaptic loss and degeneration of basal forebrain cholinergic neurons. Importantly, trisomic mice have deficits in spatial and non-spatial learning and memory linked to the septohippocampal connectome including CA1 pyramidal neurons. RNA-seq results in 11 month old Ts2 mice compared to normal disomic (2N) littermates indicate medial deep and superficial CA1 neurons exhibit unique gene expression profiles, which also differ from pooled CA1 pyramidal neurons collected along the medial-lateral axis. Classes of transcripts with significant differences in Ts2 compared to 2N CA1 pyramidal neurons that were unique to deep or superficial expression included apoptotic genes and cell signaling factors. By examining these distinct transcriptomic differences, we can potentially link innervation differences to gene expression and changes in canonical pathway expression. Preliminary RNA-seq profiling results support the hypothesis both deep and superficial CA1 pyramidal neurons have dysregulated gene

expression in Ts2 mice. We are undertaking bioinformatic inquiry (e.g., IPA, KEGG, and GO) to examine mechanistic pathways and biological functions that may be relevant towards deciphering the pathogenesis of DS and AD. Understanding expression profiles of distinct cell populations involved in a key vulnerable learning and memory circuit may translate into novel treatment options for DS and possibly lead to therapeutics for arresting cognitive decline associated with AD.

Disclosures: M.J. Alldred: None. **S.C. Penikalapati:** None. **S. Lee:** None. **A. Heguy:** None. **S.D. Ginsberg:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.05

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

Support: P01-AG066597
R01-NS109260
R01-AG066152

Title: Shared and disparate genotype contributions to TDP-43 related phenotypes

Authors: *B. SPENCER[1], D. IRWIN[2], V. M. VAN DEERLIN[3], C. MCMILLAN[1]; [1]Neurol., [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Dept Pathol & Lab. Med., Univ. Pennsylvania, Philadelphia, PA

Abstract: Case-control genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with risk of neurodegenerative diseases related to TAR DNA-binding protein (TDP-43) proteinopathies, including amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and hippocampal sclerosis of aging (HS-Aging). However, it is unclear the extent to which risk SNPs are shared or disparate across TDP-43 proteinopathies and whether these genotypes contribute to distinct phenotype presentations. We selected 69,360 SNPs that were common across prior case-control GWAS summary statistics for ALS, FTLD with TDP-43 inclusions (FTLD-TDP), and HS-Aging risk and exceeded a minimum threshold of risk ($p<0.05$) for at least one phenotype. We then performed a weighted correlation analysis to identify modules of highly correlated SNPs. This revealed 6 distinct modules including 3 modules strongly associated with either ALS (containing 19,912 SNPs), FTLD-TDP (containing 19,717 SNPs), or HS-Aging (containing 16,424 SNPs) and 3 that were strongly associated with phenotypic pairs, ALS-FTLD (containing 3,717 SNPs), ALS-HS-Aging (containing 4,888 SNPs), and FTLD-HS-Aging (containing 4,702 SNPs). To establish whether these modules confer biological meaning, we evaluated the associations between module SNPs

and gene expression quantitative trait loci (eQTL) from the public Genotype-Tissue Expression project for vulnerable brain regions associated with these phenotypes: spinal cord, frontal cortex, and hippocampus. To test whether modules contained a significant proportion of eQTLs we compared actual SNP-gene pairs within a region relative to 1000 bootstrap samples of pseudo-module assignments for each SNP-gene pair. Several biologically plausible regional differences were observed. Within the frontal cortex and spinal cord, but not the hippocampus, there were more SNP-gene pairs in the module representing the overlap between ALS and FTLD-TDP than in the pseudo-module (p= 0.04 and 0.04, respectively). In hippocampus, but not frontal cortex or spinal cord, there were more SNP-gene pairs in the module representing the overlap between HS-Aging and FTLD-TDP (p= 0.02). Examining genotypes revealed modules of both shared and disparate correlated SNPs across phenotypes associated with TDP-43 proteinopathies which have biologically plausible regional eQTL associations. We suggest that genetic variation across TDP-43 proteinopathies may contribute to the phenotypic heterogeneity across these neurodegenerative diseases related in part to transcriptomic signatures of selective vulnerability in the brain.

Disclosures: B. Spencer: None. **D. Irwin:** None. **V.M. Van Deerlin:** None. **C. McMillan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Dr. McMillan receives a research grant from Biogen, Inc. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); He also receives an honorarium as Associate Editor of NeuroImage: Clinical. F. Consulting Fees (e.g., advisory boards); Dr. McMillan provides consulting services for personal fees from Invicro and Axon Advisors on behalf of Translational Bioinformatics, LLC.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.06

Topic:

Support: R01AG071682
R21AG059177
R21AG055964

Title: ApoE2 reverses glycolytic deficit in ApoE4 models

Authors: *X. ZHANG[1], *X. ZHANG[2], H.-J. MOON[2], T. SIAHAAN[3], L. ZHAO[2,4];
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Abstract: Continued clinical failures in the search for treating Alzheimer's disease (AD) raise questions about the validity of currently focused therapeutic targets, underscoring the importance of an alternative concept that emphasizes on the neuroprotective mechanism to promote brain resilience against AD. We recently demonstrated that human ApoE genetic isoforms (ApoE2, ApoE3, ApoE4) differentially modulate neuronal glycolytic metabolism with the ApoE2-expressing cells exhibiting the most robust while the ApoE4-expressing cells display the most deficient profile. Our follow-up study revealed that these ApoE isoforms-mediated glycolytic differences directly correlated to cellular overall health status, as indicated by both morphological phenotype and metabolic activity of the cells. In the present study, we investigated glycolytic changes in cells stably expressing hApoE4 upon a transient transfection of pCMV-hApoE2. Results show that 48hr post-transfection of hApoE2 induced a significant increase in both the expression and enzymatic activity of hexokinase (HK) when compared to the vehicle-treated group. Furthermore, we treated human ApoE4 knock in (hApoE4-KI) mouse primary cortical neurons with recombinant human ApoE2 (rhApoE2) protein for 4 days and found that exposure to rhApoE2 protein led to a considerable upregulation of HK2 and synaptic markers in hApoE4-KI primary neurons. Interestingly, a significant decrease of endogenous hApoE4 protein level was also observed in rhApoE2 protein treated primary neurons. We next examined the ability of rhApoE2 protein to improve the survival of hApoE4KI primary cortical neurons exposed to H_2O_2 for 30mins. Data indicate that rhApoE2 treatment effectively protected primary neurons against H_2O_2 insult assessed by less released LDH, potentially via the upregulation of HK. In our further *in vivo* study, we delivered rhApoE2 protein using ADTC5 peptide as a blood brain barrier modulator into the brains of hApoE4KI mice via tail vein injection. Results showed a remarkable higher deposition of rhApoE2 protein compared to vehicle treated groups, indicating an enhanced delivery of rhApoE2 protein into the brain. In addition, a significant increase of HK was observed in the brains treated with rhApoE2 protein and ADTC5, with no significant change of GFAP level, indicating that introduction of rhApoE2 via ADTC5 can potentially reverse the glycolytic deficit in ApoE4 brains without inducing neurotoxicity. These data provide a proof of concept for the potential of translating ApoE2-mediated neuroprotective mechanisms into a novel therapeutic approach in the prevention and early intervention of AD.

Disclosures: X. Zhang: None. **H. Moon:** None. **T. Siahaan:** None. **L. Zhao:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.07

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

Support: NIH Grant R21AG055964

NIH Grant R21AG059177
NIH Grant R01AG071682

Title: Brain mitochondrial clusterin (mitoCLU) and its potential role in the regulation of F1Fo-ATP synthase

Authors: *H.-J. MOON[1], S. K. HERRING[1], P. RAWAL[1], L. ZHAO[1,2];
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Abstract: Clusterin (CLU), or apolipoprotein J (ApoJ), is currently assessed as the third most predominant genetic risk factor for the development of late-onset Alzheimer's disease (LOAD). A number of single nucleotide polymorphisms (SNPs) in CLU, with the majority being intronic, have been linked to significantly altered LOAD risk; however, it is unclear how these SNPs affect CLU mRNA and protein isoform expression and function in the brain. We have discovered that the unglycosylated, non-secreted mitochondrial isoform of CLU protein (mitoCLU) is localized to the mitochondrial matrix and expressed in both rodent and human neurons and astrocytes. To understand the neural function of mitoCLU, we performed mitoCLU coimmunoprecipitation and proteomic analysis using mitochondrial fractions isolated from human neuronal cell lines. We identified several potential mitoCLU-interacting proteins, including two crucial subunits of mitochondrial F1Fo-ATP synthase, a key enzyme for mitochondrial oxidative phosphorylation and ATP synthesis. In addition, we evaluated the impact of mitoCLU on mitochondrial respiration by performing a mito stress test. MitoCLU significantly increased the mitochondrial spare respiratory capacity and total ATP production. Taken together, these findings suggest that mitoCLU may play an important role in the regulation of the activity of mitochondrial F1Fo-ATP synthase, and which may be negatively affected by CLU variants leading to the development of risk phenotype for LOAD.

Disclosures: H. Moon: None. **S.K. Herring:** None. **P. Rawal:** None. **L. Zhao:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.08

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

Support: NIH Grant R21AG055964
NIH Grant R21AG059177
NIH Grant R01AG071682

Title: Regulation of astrocyte-microglia crosstalk and neuroinflammation by clusterin

Authors: P. RAWAL[1], H.-J. MOON[1], L. ZHAO[1,2];
[1]Pharmacol. and Toxicology, Sch. of Pharm., [2]Neurosci. Grad. Program, Univ. of Kansas, Lawrence, US-KS, KS

Abstract: Clusterin (CLU), also known as apolipoprotein J, is one of the top three genetic risk factors for the development of late-onset Alzheimer's disease (LOAD); however, how its genetic variants influence the risk of LOAD is unknown. Apart from the well-characterized amyloid and tau pathologies, the presence of extensive neuroinflammation mediated by microglia, the brain-resident immune cells, has been emerging as a major contributor in the pathogenesis of LOAD. Therefore, modulation of microglial activation is crucial for retaining microglial homeostasis and maintaining overall brain health. In this study, using mouse primary astrocytic and microglial cultures as well as a microglial cell line, we analyzed the expression profile of CLU and the role of CLU on microglial response to inflammatory challenge induced by lipopolysaccharide (LPS). We found that CLU is abundantly expressed in and secreted from astrocytes, however, it is neither expressed in nor secreted from microglia. Furthermore, both astrocyte-secreted and recombinant CLU glycoprotein interacted with microglia and reduced the level of LPS-induced microglial inflammation. We have also identified a crosstalk between astrocytes and microglia in which LPS induces microglial inflammation, which further induces astrocytic activation that results in increased synthesis and secretion of CLU. In addition to the immunomodulatory role of CLU in vitro, we found that the loss of CLU can lead to a chronic neuroinflammatory state indicative of AD risk in vivo. Neuroinflammation induced by intraperitoneal injection of LPS was also found to be exacerbated in CLU knockout mice when compared to wild type mice. Furthermore, consistent with our in vitro studies, LPS induced neuroinflammation resulted in an increased expression of CLU in the brain in WT mice. Overall, these findings indicate that CLU may play an important immunomodulatory and anti-inflammatory role in the brain.

Disclosures: P. Rawal: None. **H. Moon:** None. **L. Zhao:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.09

Topic:

Support: Fisher Center for Alzheimer's Research Foundation
NIA 1RF1AG054564
Cure Alzheimer's fund
H2020-MSCA-IF-2017 (GA: 799638)

Title: Dek proto-oncogene: a new modulator of selective neuronal vulnerability in Alzheimer's disease.

Authors: *P. RODRIGUEZ-RODRIGUEZ[1,2], I. SALAS-ALLENDE[2], V. YAO[3], W. WANG[2], Z. PLAUTZ[2], A. BARNEA-CRAMER[2], S. SINHA[2], P. GREENGARD[2], O. TROYANSKAYA[4], A. CEDAZO-MINGUEZ[1], M. FLAJOLET[2], J.-P. ROUSSARIE[2,5];
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Abstract: Excitatory neurons of the layer II of lateral entorhinal cortex (ECII) are the first neurons to degenerate in Alzheimer's disease (AD). They develop neurofibrillary tangles (NFT, intracellular aggregates of hyperphosphorylated tau protein) at prodromal AD stages, and their degeneration correlates with the clinical symptoms observed with the disease progression. To identify potential drivers of AD pathology in ECII neurons, we reprioritized GWAS data for NFT formation by performing Network-Wide association study (NetWAS 2.0). This analysis identified DEK proto-oncogene as a potential driver of tau pathology in ECII neurons. Our results show that downregulation of *Dek* in ECII neurons *in vitro* and *in vivo* and leads to alterations in synaptic transmission, neuronal plasticity and learning and memory associated pathways. Interestingly, while *Dek* silencing leads to increased chromatin accessibility in the promoter and proximal enhancer region of the immediate early gene *Egr1*, its expression levels are overall decreased in *Dek*-silenced neurons. Induction of neuronal depolarization by KCl leads to a higher induction of *Egr1* in *Dek*-silenced neurons compared to control, supporting the notion that *Egr1* downregulation is associated with changes in neuronal excitability. Furthermore, these alterations are accompanied by deregulation in Tau exon 10 splicing, leading to an imbalance in the 4R/3R Tau ratio, Tau accumulation in ECII neurons *in vivo*, microglia activation and ECII neuron loss. Together, our results identify *Dek* proto-oncogene as a novel regulator of neuronal plasticity and synaptic transmission which deficiency leads to AD-associated pathological alterations.

Disclosures: P. Rodriguez-Rodriguez: None. **I. Salas-Allende:** None. **V. Yao:** None. **W. Wang:** None. **Z. Plautz:** None. **A. Barnea-Cramer:** None. **S. Sinha:** None. **P. Greengard:** None. **O. Troyanskaya:** None. **A. Cedazo-Minguez:** None. **M. Flajolet:** None. **J. Roussarie:** None.

Poster

P193. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P193.10

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

Support: Cure Alzheimer's Fund
Fisher Center for Alzheimer's Research Foundation
NIA Grant 1RF1AG054564

Title: Molecular Atlas of Alzheimer's Disease Vulnerable Entorhinal Cortex

Authors: W. WANG[1], P. RODRIGUEZ-RODRIGUEZ[1,2], I. SALAS-ALLENDE[1], M. FLAJOLET[3], ***J.-P. ROUSSARIE**[4,1];
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Abstract: A major obstacle in our understanding of early stages of Alzheimer's disease (AD), is our limited knowledge of the very first neurons to die in the disease, and of their surrounding cellular environment. In most patients with amnestic AD, neurons from the layer II of the entorhinal cortex (ECII) are the first neurons to form neurofibrillary tangles and degenerate. We previously profiled neurons from the entire ECII in the mouse using cell-type specific ribosomal profiling and demonstrated that a gene module involved in axonal plasticity underlies their vulnerability to neurofibrillary degeneration (Roussarie et al., Neuron, 2020). This proved that molecular characteristics of ECII neurons could predispose them to the formation of pathological lesions in AD. A large literature describes the heterogeneity in connectivity, cytoarchitectonics and function of this region in mice and primates. In addition, the transentorhinal subdivision of the EC shows strikingly higher vulnerability than the rest of the ECII. We now propose to more precisely identify the molecular subtypes that compose this layer. Using single-nucleus RNA-sequencing and *in situ* hybridization in the mouse, we 1) delineate molecular subpopulations of EC - some discrete, some in transcriptional gradients, in a data-driven way, 2) identify markers for these anatomically distinct populations of ECII neurons, as well as for specific cell states within these populations, 3) identify subpopulation-specific signal in our whole ECII ribosomal profiling data. Overall this study is an indispensable first step in the characterization of transentorhinal neurons, and in the precise molecular dissection of the neuronal pathological cascade taking place during prodromal AD.

Disclosures: W. Wang: None. **P. Rodriguez-rodriguez:** None. **I. Salas-Allende:** None. **M. flajolet:** None. **J. Roussarie:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH T32 NS105607
NIH T32 HG000046
NIH F31 NS122297

Title: Identifying Cellular and Molecular Mechanisms Underlying Selective Regional Degeneration in Spinocerebellar Ataxia Type 2

Authors: *A. B. ROBBINS[1,3], P. T. RANUM[3], B. L. DAVIDSON[3,2];
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Abstract: Many genes implicated in neurodegenerative disorders are widely expressed yet trigger dysfunction in specific brain regions and cell populations. Here we investigate the molecular underpinnings of selective degeneration in a fatal, late-onset disorder, Spinocerebellar Ataxia Type 2 (SCA2). SCA2 is caused by an abnormal CAG repeat expansion in the *ATXN2* gene, conferring a toxic gain of function. Clinical manifestations of SCA2 are linked to cerebellar dysfunction, including speech difficulties and loss of motor coordination. Although *ATXN2* is ubiquitously expressed, cerebellar Purkinje cells are most vulnerable to degeneration and cell death. Clinical case studies of SCA2 neuropathology demonstrate a distinct anterior to posterior atrophy pattern and sparing of the most posterior cerebellum. The mechanisms underlying both the variable cerebellar loss and Purkinje cell-specific vulnerability have not yet been investigated**.** Recent transcriptional profiling of individual cerebellar lobules has identified variation in specialized neuronal and glial subtypes across the adult wild-type murine cerebellum. Here we sought to identify differential expression of disease-relevant pathways contributing to regional and cell type-specific cerebellar vulnerability in the context of SCA2 and normal cerebellar aging. Utilizing a well-characterized mouse model of SCA2, we profiled the cerebellar transcriptome at tissue and single-cell level while retaining spatial information across the anterior to posterior cerebellum via fine microdissection. Tissue for single nuclei RNA-sequencing (snRNA-Seq) was collected at pre-symptomatic, early, and late-stage disease time points, capturing transcriptional alterations across the spatiotemporal landscape of SCA2 pathogenesis. To overcome the cost and sample size limitations of prevailing droplet-based snRNA-seq methodologies, we implemented SPLiT-Seq. This barcode-based single cell RNA-Seq workflow facilitates the creation of highly multiplexed snRNA-Seq datasets. By encoding spatiotemporal and sample ID information within three rounds of combinatorial barcoding, we indexed 96 biological samples per sequencing library, simultaneously assessing samples from distinct cerebellar regions, genotypes, and timepoints. This study is the first to characterize SCA2 degeneration and disease progression at a single-cell spatiotemporal resolution, alongside cerebellar aging in normal and disease brain. Our results offer new insights into the cell-type-specific contributions to regional disease vulnerability and the spatial regulation of genes and pathways for future therapeutic validation.

Disclosures: A.B. Robbins: None. **P.T. Ranum:** None. **B.L. Davidson:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS114882

Title: Translational profiling of striatal spiny projection neurons in a mouse model of dystonia reveals distinct adaptations in D1 dopamine receptor- versus D2 dopamine receptor-expressing cells

Authors: *K. M. ROMAN, E. J. HESS;
Emory Univ., Atlanta, GA

Abstract: Striatal dysfunction is implicated in many forms of dystonia with diverse etiologies. The striatum is primarily composed of spiny projection neurons (SPNs), which generally express either D1 or D2 dopamine receptors (D1Rs; D2Rs). D1R- and D2R-expressing SPNs act in concert to regulate motor activity by modulating basal ganglia output. Although both D1R- and D2R-expressing SPNs are implicated in dystonia, our knowledge of the cell type-specific molecular abnormalities underlying dystonia is extremely limited. We have previously demonstrated that the striatum is central to the dystonia in a mouse model of DOPA-responsive dystonia (DRD). Further, both D1R and D2R signaling mediates dystonia in DRD mice. Thus, this novel mouse model provides an opportunity to understand striatal cell-type specific molecular adaptations in dystonia. We used translating ribosome affinity purification (TRAP) to purify cell-type specific mRNA from either D1R- or D2R-expressing SPNs from DRD and control littermates and used RNA-seq to obtain an extensive profile of the actively translating genes. In D1R-expressing SPNs, we detected 31,549 unique transcripts, and 806 transcripts were differentially expressed (451 upregulated; 355 downregulated) in DRD compared to control mice (Benjamini-Hochberg padj<0.05; |FC|≥1.5; n=3F/5M mice/genotype). In D2R-expressing SPNs, we detected 28,530 unique transcripts and 932 transcripts were differentially expressed (496 upregulated; 436 downregulated) in DRD compared to control mice (Benjamini-Hochberg padj<0.05; |FC|≥1.5; n=5F/3M mice/genotype). In DRD mice, a higher percentage of genes were differentially expressed in D2R-SPNs (3.27%) than in D1R-SPNs (2.55%). Only 121 transcripts were differentially expressed in both SPN subtypes (38 commonly upregulated; 15 commonly downregulated; 68 oppositely regulated). We used Gene Set Enrichment Analysis to identify dysregulated biological pathways that may play an important role in the pathomechanisms of dystonia and found that MAPK signaling pathway genes are abnormally regulated in D1R-SPNs. In contrast, calcium signaling pathway genes are abnormally regulated in D2R-SPNs. Interestingly, several differentially expressed genes from D1R-SPNs (6 genes) and D2R-SPNs (5 genes) are already implicated in dystonia. Identifying dysregulated pathways in D1R- and D2R-expressing SPNs may provide a foundation for the development of desperately needed therapeutics for the many dystonias associated with striatal dysfunction.

Disclosures: K.M. Roman: None. **E.J. Hess:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: ARSACS Foundation

Title: Loss of the ataxia protein sacsin perturbs trafficking, formation of cell adhesions, and disrupts synaptic organization of Purkinje neurons

Authors: L. E. ROMANO[1], W. AW[2], K. M. HIXSON[2], T. HAVENER[2], S. HOWELL[2], T. NOVOSELOVA[1], C. HALL[1], P. CHAPPLE[1], ***J. WOLTER**[2];
[1]Queen Mary Univ. of London, London, United Kingdom; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset cerebellar ataxia that is caused by mutations in the gene *SACS*. The ARSACS mouse model and patient derived cell lines display mitochondrial dysfunction and abnormal neurofilament structure, yet the large size of the SACS protein (520 kDa) has hampered standard biochemical characterization. Thus, it is unclear how the loss of SACS causes these deficits, or why they manifest as a cerebellar ataxia. To address this critical gap in knowledge, we employ a multi-omics approach to characterize molecular deficiencies in *SACS* knockout (KO) cells, including phosphoproteomics, kinome profiling, cell surface proteomics, RNA-seq, and co-IP mass spectrometry. We identified overabundance and hyperphosphorylation of the protein Tau in *SACS* KO cells, as well as deficits in microtubule structure, dynamics, and protein trafficking. We also identified altered membrane localization of multiple synaptic adhesion proteins, including ITGA1, NRCAM, NFASC, and CNTN1. Mutations in several of these genes cause various cerebellar ataxias with overlapping phenotypic manifestations with ARSACS. We also identified striking synaptic deficits in the deep cerebellar nucleus of the ARSACS mouse, prior to the onset of Purkinje cell death or motor deficits. In all, our data suggests that deficits in synaptic adhesion protein trafficking is a causal molecular deficiency in ARSACS.

Disclosures: L.E. Romano: None. **W. Aw:** None. **K.M. Hixson:** None. **T. Havener:** None. **S. Howell:** None. **T. Novoselova:** None. **C. Hall:** None. **P. Chapple:** None. **J. Wolter:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NS083706
NS088321
MH119803
AG066447
NS041228

Title: Differential effects of enhanced Wnt-β-catenin signaling in Purkinje cells and glial cells in SCA1

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Abstract: Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disorder characterized by progressive ataxia and degeneration of specific neuronal populations, including Purkinje cells (PCs) in the cerebellum. SCA1 is caused by a nucleotide expansion of a glutamine-encoding (CAG) repeat tract in *ATXN1*, and can be characterized by progressive ataxia, mild cognitive impairments, and ultimately, respiratory failure, etc. Previous studies have demonstrated a critical role for various evolutionarily conserved signaling pathways in cerebellar patterning, such as the Wnt-β-catenin pathway; however, the roles of these pathways in adult cerebellar function and cerebellar neurodegeneration are largely unknown. In this study, we found that Wnt-β-catenin activity was progressively enhanced in several cell types in the adult SCA1 mouse cerebellum, and that activation of this signaling occurs in an Ataxin-1 polyglutamine (polyQ) expansion-dependent manner. Genetic manipulation of the Wnt-β-catenin signaling pathway in specific cerebellar cell populations revealed that activation of Wnt-β-catenin signaling in PCs alone was not sufficient to induce SCA1-like phenotypes, while its activation in astrocytes including Bergmann glia (BG) resulted in gliosis and disrupted BG localization, which was replicated in SCA1 mouse models. The role of BG in degeneration of PCs in SCA1 is currently under further investigation, as well as the necessity of Wnt-β-catenin signaling activation in BG towards SCA1 pathology. Our studies identify a novel mechanism in which polyQ-expanded Ataxin-1 positively regulates Wnt-β-catenin signaling, and demonstrate that different cell types have distinct responses to the enhanced Wnt-β-catenin signaling in the SCA1 cerebellum, underscoring an important role of astrocytes, specifically BG, in SCA1 pathogenesis.

Disclosures: K. Luttik: None. **L. Tejwani:** None. **H. Ju:** None. **T. Driessen:** None. **C. Smeets:** None. **J. Lim:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant DK116624
NIH Grant MH115673
NIH Grant MH113352
Roy J. Carver Charitable Trust
Iowa Neuroscience Institute
Jordan's Guardian Angels

Title: Genetically altering mitochondrial morphology does not modify the progression of symptoms in a mouse model of ARSACS

Authors: *C. CHEN[1], R. A. MERRILL[2], Y. KONG[1], G. HEALEY[3], S. STRACK[4]; [1]Neurosci. and Pharmacol., Univ. of Iowa, IOWA CITY, IA; [2]Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA; [3]Neurosci. and Pharmacol., Univ. of Iowa, Iowa, IA; [4]Neurosci. and Pharmacol., Univ. Iowa Col. Med., Iowa City, IA

Abstract: BACKGROUND: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disease caused by a loss-of-function mutations in the SACS gene. ARSACS is characterized by cerebellar dysfunction and atrophy resulting from Purkinje cell death. Mice lacking the sacsin protein, encoded by SACS gene, recapitulate the human disease with age-dependent neurodegeneration of cerebellar Purkinje cells. Additionally, loss of sacsin function may lead to hyperfused mitochondria that accumulate in the soma and leads to the Purkinje neuron loss.**METHODS:** Since mitochondrial morphology may underlie disease progression, we have crossed the sacsin knockout mouse with two additional knockout mice that either increase or decrease mitochondrial fission. A Kinase Anchoring Protein 1 (AKAP1) loss is known to induce mitochondrial fission while loss of the protein phosphatase 2A (PP2A) regulator Bbeta2 is known to decrease mitochondrial fission. These knockout mice elicit their effects through modifying the mitochondrial fission mediator dynamin-related protein 1 (Drp1). **RESULTS:** Preliminary results show no change in the age-related progression of the sacsin KO mice phenotypes associated with cerebellar neurodegeneration following crosses with either AKAP1 KO mice or Bbeta2 KO mice. Behavioral tests utilized include rotorod and balance beam. **CONCLUSION:** Dysfunctional mitochondrial fission may underlie the neurodegeneration of cerebellar Purkinje cells. By modifying the fission mediator Drp1 through genetic intervention, we were not able to alter the progression of a mouse model of the disease. Additional work will include defining the mitochondrial morphology and function in these new mouse crosses.

Disclosures: C. Chen: None. **R.A. Merrill:** None. **Y. Kong:** None. **G. Healey:** None. **S. Strack:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Ataxia Foundation SCA Young Investigator Award
NIH Grant T32-NS007222-33
NIH Grant U01-NS106670
NIH Grant R01-NS38712
NIH Grant R01-NS122751

Title: Deficits in oligodendrocyte differentiation foundearlyin SCA3 disease pathogenesis

Authors: K. H. SCHUSTER[1], A. J. ZALON[1], D. DIFRANCO[1], N. STEC[1], H. ZHANG[1], S. JARRAH[1], Y. GUAN[1], H. L. PAULSON[2], *H. MCLOUGHLIN[1];
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Abstract: Spinocerebellar Ataxia Type 3 (SCA3) is the most common dominantly inherited ataxia. This progressive, neurodegenerative disease is caused by a CAG repeat expansion in the *ATXN3* disease gene, which produces an enlarged poly-glutamine repeat in the ATXN3 protein. Research of the pathogenic mechanisms underlying SCA3 has mainly focused on the gain-of-toxic functions of mutant ATXN3 (mutATXN3) that lead to neuronal impairment and death. Consistent with evidence across other neurodegenerative diseases, our studies demonstrate that oligodendrocytes (OLs) play a major role in SCA3 pathogenesis. By mapping transcriptional changes in the most vulnerable SCA3 brain regions across disease states in transgenic mice, we revealed an explicit dysfunction in OL maturation. This phenomenon was seen earliest and most prominently in the brainstem relative to the cerebellum. These differentially expressed genes were verified by biochemical analysis, revealing a decrease in immature OL genes and an increase in mature OL genes. We also utilized SCA3 mice crossed with Mobp-eGFP reporter mice to evaluate mature OL counts in the brain regions of interest. We confirmed a significant loss of mature OLs by 16 weeks in the pons and deep cerebellar nuclei. However, mature OL counts in the corpus callosum, a white matter tract that expresses mutATXN3 but is not affected in SCA3, displayed no difference between diseased mice and WT littermates. This indicates OL maturation deficits reflect the regional specificity of degeneration in SCA3. Notably, we found no changes in OL lineage cell counts from SCA3 vulnerable brain regions using a pan-OL antibody. With these data, we hypothesize a stalling of OL maturation in disease due to mutATXN3 expression in SCA3 vulnerable brain regions. Importantly, we confirmed that ATXN3 knockout mice show no biochemical or histological changes in OL expression at

analogous timepoints. In summary, our data suggest a severe deficit in OL maturation caused by the gain-of-toxic function of mutATXN3 early in SCA3 disease progression.

Disclosures: K.H. Schuster: None. **A.J. Zalon:** None. **D. DiFranco:** None. **N. Stec:** None. **H. Zhang:** None. **S. Jarrah:** None. **Y. Guan:** None. **H.L. Paulson:** None. **H. McLoughlin:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.07

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: The Children's Brain Disease Foundation

Title: Transient changes in choroid plexus function during asymptomatic period in CLN2 mouse model

Authors: *M. S. DOMOWICZ, N. A. PEREZ CATALAN, P. CLAUDIO-VAZQUEZ, T. GONZALEZ, Y. MARTINEZ-CASTILLO, N. B. SCHWARTZ;
Dept. of Pediatrics, Univ. of Chicago, Chicago, IL

Abstract: Neuronal ceroid lipofuscinosis type 2 (CLN2 disease) is a neurodegenerative disease that results in progressive visual deterioration resulting in blindness, intellectual and motor decline, epilepsy, ataxia, spasticity, and premature death. Here we are using a well characterized mouse model of CLN2, in which the tripeptidyl peptidase 1 (*Tpp1*) gene is disrupted by gene targeting resulting in loss of detectable *TPP1* activity leading to progressive neurological phenotypes including; ataxia, decrease motor function and early death (median survival of 19 weeks) (Sleat D.E. et al., 2004). Global RNAseq analysis of 1- to 4-month-old *Tpp1-/-* mouse forebrain/midbrain and cerebellum show a transient increase in transcripts expressed almost exclusively in the choroid plexus (ChP) during asymptomatic stages of the disease (1- to 2-month-old). These initial transcriptional changes in the ChP include genes for structural extracellular matrix proteins, secreted proteins, transporters, tight junction components, receptors, integral components of secretory epithelium membrane, cilia components and enzymes, all of which indicate a global functional activation of the ChP. To confirm that these changes were associated with the ChP, we assessed expression levels in ChP isolated from the lateral and fourth ventricle by qPCR, and verified increased transcriptional expression of *Aqp1, Clic6, Kl, Prlr, Ttr*, and the ciliary dynein *Dnah1 and Dnah11* in the *Tpp1-/-* mouse ChP. Also, immunohistochemical analysis using the ciliary marker, acetylated tubulin, indicated an increase in the number of cilia per cell in ChP epithelium. Transcriptional upregulation of *Fzd4* and downregulation of *Axin2*, as well as redistribution of beta-catenin to the apical surface of the ChP epithelium were also observed in the *Tpp1-/-* ChP, indicating that the Wnt/beta-catenin pathway

may be an important regulatory driver of the ChP response in affected brains. Together, our results provide novel insights into early events in the onset of CLN2 disease that involve functional and structural changes in ChP, a highly critical brain structure for metabolite recycling.

Disclosures: M.S. Domowicz: None. **N.A. Perez Catalan:** None. **P. Claudio-Vazquez:** None. **T. Gonzalez:** None. **Y. Martinez-Castillo:** None. **N.B. Schwartz:** None.

Poster

P280. Ataxia and Dystonia: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P280.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR grant

Title: Characterizing the binding of bupropion and ibogaine to the dopamine transporter

Authors: *E. WILLIAMS[1], A. SALAHPOUR[1], M. SHAPIRA[1,2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Structural Genomics Consortium, Toronto, ON, Canada

Abstract: Individuals afflicted with Dopamine Transporter Deficiency Syndrome (DTDS) have minimal to no dopamine transporter (DAT) proteins at plasma membranes. Pharmacological chaperones selectively binding the DAT present a unique and viable path for treatment of select DTDS disease-causing variants. Two such compounds have been previously identified: bupropion and ibogaine, both atypical DAT inhibitors. However, it is not well understood how these drugs bind to the orthosteric binding site of DAT. Characterizing the binding of bupropion and ibogaine to DAT will provide critical information about the nature of the DAT orthosteric binding site and will aid in identifying more efficacious and potent pharmacological chaperones for treatment of DTDS. We used molecular homology modeling of the human DAT to model and investigate the binding of bupropion and ibogaine to DAT. Currently, the predicted binding site and key residues involved in the binding of bupropion and ibogaine are being explored using site-directed mutagenesis followed by assessment of inhibition of binding and chaperone activity of the compounds. Our studies will provide key information as to which residues are necessary for bupropion and ibogaine binding and pharmacological chaperone activity.

Disclosures: E. Williams: None. **A. Salahpour:** None. **M. Shapira:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.01

Topic: C.06. Neuromuscular Diseases

Support: NIH grant R21NS102599
NIH grant R01NS089640

Title: Nr1d1 down-regulation in astrocytes is detrimental to co-cultured motor neurons

Authors: K. M. KILLOY[1], B. A. HARLAN[3], M. PEHAR[2], ***M. R. VARGAS**[1];
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Abstract: Nuclear receptor subfamily 1 group D member 1 (Nr1d1, also known as Rev-erbα) is a nuclear transcription factor part of the molecular clock that encodes circadian rhythms and may link daily rhythms with metabolism and inflammation. Nr1d1, unlike most nuclear receptors, lacks a ligand-dependent activation function domain 2 and is a constitutive transcriptional repressor. We have previously shown dysregulated clock and clock-controlled genes expression in multiple tissues from amyotrophic lateral sclerosis (ALS) mouse models. ALS is the most common adult-onset motor neuron disease, caused by the progressive degeneration of motor neurons in the spinal cord, brain stem, and motor cortex. Approximately 10-20% of familial ALS is caused by a toxic gain-of-function induced by mutations of the Cu/Zn-superoxide dismutase (SOD1). Here we explore Nr1d1 dysregulation in the spinal cord of mutant hSOD1-linked ALS-mouse models and its consequences on astrocyte-motor neuron interaction. Nr1d1 protein and mRNA expression is significantly downregulated in the spinal cord of symptomatic mice expressing mutant hSOD1, while no changes were observed in age-matched animals over-expressing wild-type hSOD1. In addition, Nr1d1 down-regulation in primary astrocytes causes a pro-inflammatory phenotype and decreases the survival of co-cultured motor neurons. Nr1d1 orchestrates the crosstalk between physiologically pathways identified to be disrupted in ALS (e.g. metabolism, inflammation, redox homeostasis, and circadian rhythms) and we observed that down-regulation of Nr1d1 alters astrocyte-motor neuron interaction. Our results suggest that Nr1d1 could be a potential therapeutic target to prevent astrocyte-mediated motor neuron toxicity in ALS.

Disclosures: K.M. Killoy: None. **B.A. Harlan:** None. **M. Pehar:** None. **M.R. Vargas:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.02

Topic: C.06. Neuromuscular Diseases

Support: Target ALS Consortia
Frick Foundation for ALS research
NINDS and NIA (R01NS117461)
DoD (W81XWH-21-1-0082)
Mayo Clinic Center for Biomedical Discovery

Title: Jnk promotes stress granule assembly by epigenetically regulating g3bp1

Authors: *T. SAHANA, K. ZHANG;
Mayo Clin., Jacksonville, FL

Abstract: Stress granules (SGs), RNA/protein condensates assembled in cells upon stress, can trigger the aggregation of their component proteins TAR DNA-binding protein-43 (TDP-43), fused in sarcoma (FUS), etc. Since aggregation of these proteins is a pathological hallmark of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD), two related neurodegenerative diseases, SGs are considered the crucible of these diseases. Given this critical role, SGs have been actively studied in the ALS/FTD research field. Biochemical studies have revealed the molecular mechanism by which they assemble, and drug screens have identified their inhibitors. However, how SG assembly is regulated at the cellular level is unclear—an important question to both fundamental cell biology and disease pathophysiology.
In a genetic screen, we identified that an RNAi against the *basket*, the fly homolog of c-Jun N-terminal kinase (JNK), suppresses neurodegeneration in a *Drosophila* model of *C9ORF72*-mediated ALS and FTD (c9ALS/FTD), the most common familial ALS/FTD. Consistent with these data, JNK activity is upregulated in both c9ALS/FTD *Drosophila* and U-2 OS cell models. Furthermore, this upregulation is caused by ER stress activating inositol requiring enzyme 1 (IRE1). Interestingly, we found that JNK activation induces SG assembly via phosphorylation of histone 3 protein at serine 10 (H3S10), an epigenetic modification that transactivates a key SG protein, G3BP1. Consistent with these data, we showed that IRE1 or JNK inhibitors suppress H3S10 and G3BP1 levels, SG assembly, and neurodegeneration in c9ALS/FTD U-2 OS models or patient neurons derived from induced pluripotent stem cells (iPSNs). Together, our findings connected ER stress, JNK activation, epigenetic modulation, and SG assembly in a unified pathogenic pathway in c9ALS/FTD, thereby allowing us to better understand how cells respond to stress and how this process is implicated in neurodegeneration.

Disclosures: T. Sahana: None. **K. Zhang:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.03

Topic: C.06. Neuromuscular Diseases

Support: NIH R37 NS054154
The Paul E. Kelly Foundation
Uplifting Athletes Young Investigator Draft
Charcot-Marie-Tooth Association

Title: In vitro modeling of Charcot-Marie-Tooth Type 2D using engineered human induced pluripotent stem cells

Authors: *T. J. HINES[1], E. L. SPAULDING[2], J. LI[1,3], P. BAIS[1], D. E. CORTES[1], R. W. BURGESS[1];
[1]The Jackson Lab., Bar Harbor, ME; [2]Mount Desert Island Biol. Lab., Bar Harbor, ME; [3]Tufts Univ., Boston, MA

Abstract: Charcot-Marie-Tooth type 2D (CMT2D) is a dominant axonal neuropathy caused by mutations in glycyl tRNA-synthetase (GARS). The GARS enzyme charges glycine onto its cognate tRNAs for translation, but like many neurodegenerative diseases, this "housekeeping gene" causes a very specific disease phenotype when mutated. We previously performed gene expression analysis using ribotagging and RNAseq to specifically examine the motor neuron transcriptome of 3 different Gars/CMT2D mouse models. This revealed signatures consistent with activation of the integrated stress response (ISR). This was confirmed by immunofluorescence for phosphorylated eIF2α, a canonical marker of the ISR, and by RNAscope ISH for upregulated ATF4 targets. Both phospho-eIF2α and ATF4 targets were restricted to alpha-motor neurons in the spinal cord. These gene expression changes, as well as the neuropathy phenotype, were abrogated by crossing Gars mutant mice with mice lacking the cell stress sensing kinase, Gcn2. This indicates that the ISR is initiated by GCN2, which itself is activated by ribosome stalling and amino acid deprivation. Additionally, Gars mutants lacking the ribosome rescue factor, Gtpbp2, had an exacerbated neuropathy phenotype. This further supports our hypothesis of ribosome stalling inducing the integrated stress response in Gars mutant mice. In order to confirm these findings in a human system, as well as further interrogating the mechanism(s) underlying ISR activation and cell-type specificity, we have used CRISPR/Cas9 genome editing in a well-characterized "healthy control" human induced pluriopotent stem cell (hIPSC) line, KOLF2-C1, to develop cell-based models of CMT2D. These engineered cells express only WT Gars, or one of two dominant Gars mutations - G240R, a common mutation in North American CMT2D patients, and ΔETAQ, a severe, early onset allele for which we also have a mouse model. Since motor neurons are such a small fraction of the total cells in the spinal cord, it is difficult to measure changes in gene expression and protein levels by conventional methods. After differentiation, these cell-based models provide a relatively pure population of motor neurons, which enables bulk analysis of the affected cell population and high throughput screening of potential therapeutics. We are currently analyzing gene expression data and markers such as phospho-eIF2α levels to confirm ISR activation in these cells. We will

then compare changes seen in motor neurons to other terminally differentiated cell types, which are unaffected in CMT2D.

Disclosures: T.J. Hines: None. **E.L. Spaulding:** None. **J. Li:** None. **P. Bais:** None. **D.E. Cortes:** None. **R.W. Burgess:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.04

Topic: C.06. Neuromuscular Diseases

Support: NINDS Competitive Postdoctoral Fellowship Awards
Intergovernmental Personnel Act agreement
Kennedy's Disease Association Research Grant

Title: Ligand-free mutant AR promotes motor neuron-related mitochondrial dysfunction in spinal and bulbar muscular atrophy

Authors: *X. FENG[1,2,3], X.-T. CHENG[4], P. ZHENG[2], Y. LI[5], J. HAKIM[3], S. Q. ZHANG[6], S. M. ANDERSON[7], K. LINASK[8], J. ZOU[8], Z.-H. SHENG[4], C. BLACKSTONE[2,9];
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Abstract: As the first uncovered CAG-repeat expansion disease, spinal and bulbar muscular atrophy (SBMA) is an adult-onset X-linked neuromuscular disorder. Its genetic cause is a CAG repeat expansion in the androgen receptor (AR) gene. The mutant AR protein with an expanded polyglutamine (polyQ) tract, encoded by pathological CAG repeats, is prone to aggregation and induces cytotoxicity upon ligand binding, leading to progressive motor neuron degeneration and muscle atrophy. SBMA exhibits mitochondrial abnormalities. Utilizing a newly developed isogenic human induced pluripotent stem cell model, we show that mitochondrial impairments, including aberrant mitochondrial depolarization, increased mitochondrial oxidative stress, and altered cristae architecture, occurred in the neurites of the differentiated motor neuron-like cells by introducing a pathological polyQ length into the wildtype AR, while these aberrations were rescued by correcting the expanded repeats in the mutant AR. Interestingly, compared with the isogenic control cells, the AR knockout motor neurons showed no significant changes in the maintenance of membrane potential, reactive oxygen species levels, and cristae architecture in

the mitochondria, suggesting that the mitochondrial damage observed in the neurites of the disease motor neurons are possibly due to a gain of function of the mutant AR. Although androgen is widely believed to play an essential role in the mutant AR-induced cytotoxicity in SBMA, here we found that, surprisingly, the mutant protein was sufficient to lead to mitochondrial dysfunction in the disease motor neurons without ligand binding, whereas the ligand treatment only aggravated such impairments. Our data indicate that different molecular mechanisms underpin the pathological polyQ-dependent, mitochondrial-targeted cytotoxicity in SBMA motor neurons, which involve both ligand-free and ligand-bound forms of the mutant AR.

Disclosures: X. Feng: None. **X. Cheng:** None. **P. Zheng:** None. **Y. Li:** None. **J. Hakim:** None. **S.Q. Zhang:** None. **S.M. Anderson:** None. **K. Linask:** None. **J. Zou:** None. **Z. Sheng:** None. **C. Blackstone:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.05

Topic: C.06. Neuromuscular Diseases

Support: R01 NS106302

Title: Mechanistic investigation of aberrant neurite outgrowth and androgen receptor transcriptional activity in spinal and bulbar muscular atrophy

Authors: *J. KARLINER, D. MERRY;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA) is a slowly progressing X-linked neuromuscular disease characterized by the degeneration of spinal cord and brainstem lower motor neurons and muscle atrophy. It is caused by a polyglutamine (polyQ) expansion in the androgen receptor (AR), a transcription factor that promotes neurite outgrowth and regeneration in neurons. In SBMA, androgen binding to polyQ-expanded AR causes its misfolding and intranuclear aggregation, resulting in reduced AR transcriptional activity and aberrant neurite outgrowth. However, the contribution of these processes to SBMA pathogenesis is unknown. This study aims to fill these gaps by studying neurite outgrowth in the presence of DNA-binding-competent and -incompetent (V581F) polyQ-expanded AR and investigating mechanisms underlying aberrant neurite outgrowth in SBMA. Based on previous findings suggesting that reduced activity of the E3 ligase anaphase promoting complex/cyclosome-CDC20 homolog 1 (APC/C^{Cdh1}) underlies aberrant neurite outgrowth in SBMA, we will investigate the role of Cdh1 by studying its metabolism, which regulates APC/C activity. Experiments were conducted using

a PC12 cell model of SBMA in which full-length AR with pathological (AR112Q/AR111Q V581F) or nonpathological (AR10Q) polyQ-repeats is expressed under the control of a tetracycline-inducible promoter. Cells were differentiated through treatment with nerve growth factor (NGF) for 6 days, induced to express AR (+Dox) and treated with the androgen dihydrotestosterone (DHT) for 96 hours, and analyzed for the number of neurite-positive cells and Cdh1 protein expression. Through the use of live-cell imaging, preliminary results demonstrate a significant decrease in the number of neurite-positive cells upon polyQ-expanded AR expression in the presence of DHT. This is true for both DNA-binding-competent and -incompetent polyQ-expanded AR, but not for normal polyQ-length AR, demonstrating a pathological phenotype independent of AR transcriptional activity. Some cells expressing polyQ-expanded AR were observed to retract their neurites during this 96-hour period. Results also show a decrease in Cdh1 protein expression following 96 hours of Dox and DHT treatment in the presence of polyQ-expanded AR, suggesting that reduced Cdh1 expression could underly aberrant neurite outgrowth in SBMA. Ongoing studies will investigate whether altered Cdh1 phosphorylation leads to its reduced protein expression. Overall, this work helps elucidate mechanisms underlying aberrant neurite outgrowth in SBMA and determine if therapeutics should preserve or inhibit normal AR functions to be most efficacious.

Disclosures: J. Karliner: None. **D. Merry:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.06

Topic: C.06. Neuromuscular Diseases

Support: NINDS K081-K08-NS094744-02
ALS Association

Title: Ataxin-2 polyQ expansions aberrantly sequester TDP-43, impair anterograde transport of neuronal RNP granules along the axon and suppress translation

Authors: *D. WIJEGUNAWARDANA, S. S. VISHAL, P. P. GOPAL;
Dept. of Pathology, Yale Sch. of Med., New Haven, CT

Abstract: Amyotrophic lateral sclerosis (ALS), a debilitating and fatal neurodegenerative disease, is characterized by aggregates of TDP-43, an RNA-binding protein (RBP) with multiple roles in post-transcriptional RNA processing. Mutations in TDP-43 and other RBPs cause familial and sporadic ALS, highlighting altered RNA metabolism as a common pathogenic mechanism. Recent studies have identified genetic interactions between TDP-43 and Ataxin-2, a RBP with a polyglutamine (polyQ) tract normally 22-23Q in length. Intermediate-length

expansions of the Ataxin-2 polyQ tract (27-33Q) confer increased risk for ALS. However, the underlying molecular mechanisms by which Ataxin-2/TDP-43 interactions increase ALS risk are unclear. We hypothesized that Ataxin-2 polyQ expansions aberrantly scaffold TDP-43, stabilize TDP-43/Ataxin-2 interactions within ribonucleoprotein (RNP) granules and disrupt their dynamic properties, increasing the propensity for pathologic transformation of TDP-43. We used live-cell confocal imaging, photobleaching and immunofluorescence experiments to study the localization, motility and interactions of TDP-43 and Ataxin-2 in rodent primary cortical neurons. Our data show that endogenous wild type Ataxin-2 colocalizes with a subset of TDP-43 positive RNP granules, which are dynamic liquid-like biomolecular condensates. While TDP-43 positive granules exhibit oscillatory or long-range motility in the mid axon, Ataxin-2 Q22 RNP granules are predominantly stationary and localize within the proximal axon. Kymographs were used to analyze the motility of RNP granules containing TDP-43 with or without Ataxin-2 of varying polyQ lengths. We found that TDP-43 granules positive for Ataxin-2 Q30 or Q39 exhibit reduced anterograde run lengths and sequester TDP-43. In addition, our photobleaching experiments show that Ataxin-2 Q30 and Q39 expansions impair TDP-43 dynamics and FRAP recovery. These data suggest that TDP-43 RNP granule transport and intramolecular motility is perturbed in neurons with Ataxin-2 polyQ expansions. Finally, we used puromycinylation assays to show that translation is suppressed in primary cortical neurons expressing Ataxin-2 polyQ expansions, compared to those expressing Ataxin-2 Q22 or RFP. Our data suggest that Ataxin-2 polyQ expansions perturb TDP-43 anterograde transport and its spatial localization, and impair translation as well. Overall, these results indicate Ataxin-2 polyQ expansions aberrantly sequester TDP-43 and may have detrimental effects on stability, localization, and translation of transcripts critical for axonal and cytoskeletal integrity, particularly in motor neuron axons.

Disclosures: D. Wijegunawardana: None. **S.S. Vishal:** None. **P.P. Gopal:** None.

Poster

P290. Motor Neuron Disease: In Vitro Studies II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P290.07

Topic: C.06. Neuromuscular Diseases

Support: NIH DP2-NS106664
New York Stem Cell Foundation

Title: Investigation of TDP-43 phosphorylation and cleavage on TDP-43 localization and nucleocytoplasmic transport using iPSC motor neurons

Authors: *M. J. ADLER[1], A. HELD[1], A. KAVUTURU[1], C. MARQUES[1], M. E. WARD[2], B. J. WAINGER[1];

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Abstract: Aggregation of the RNA binding protein TDP-43 is the major molecular hallmark of amyotrophic lateral sclerosis (ALS), a rapidly-progressing fatal disease of the motor nervous system. Cytoplasmic TDP-43 may result in loss of normal nuclear TDP-43 function as well as gain of cytoplasmic function, potentially upon aggregate formation. Aggregates typically contain phosphorylated, truncated C-terminal fragments (CTFs), but the effects of these modifications on the compartmentalization of TDP-43 are not well characterized. Overexpression of tagged TDP-43 CTF in neuroblastoma cells shows a predominantly cytoplasmic location, association with nucleoporins, and disruption of global nucleocytoplasmic transport. It is less clear whether this disruption results specifically from TDP-43 phosphorylation, mislocalization, truncation, or expression level, and to what extent other causes of nucleocytoplasmic transport impairment are sufficient to mislocalize endogenous TDP-43. While many studies correlate TDP-43 aggregation with neuronal toxicity, a recent investigation found that TDP-43 hyperphosphorylation reduced aggregate accumulation. Our study aims to examine how TDP-43 phosphorylation and the generation of CTFs affect subcellular localization and nucleocytoplasminc transport.
We have generated mCherry-tagged TDP-43 expression constructs with point mutations in the CTF converting serines to either glutamate or alanine to mimic or block phosphorylation, respectively. We have also made 25kDa truncated TDP-43 CTFs with these same modifications. We will express these constructs in human iPSC-derived motor neurons. Using previously validated shuttling proteins as reporters of nucleocytoplasmic transport integrity, we will measure how TDP-43 phosphorylation and truncation affect nucleocytoplasmic transport function and TDP-43 location. Further, casein kinases (CK) have been recognized to phosphorylate TDP-43, CK1E colocalizing with TDP-43 in sporadic ALS postmortem tissue. In addition to phosphomimetic mutations, we will assess the effects of expressing these kinases versus applying inhibitors of the kinases, thus allowing us to evaluate over-expressed versus endogenous TDP-43. These experiments will provide insight into the interactions of TDP-43 function, nucleocytoplasmic transport, and TDP-43 modifications by phosphorylation and cleavage.

Disclosures: M.J. Adler: None. **A. Held:** None. **A. Kavuturu:** None. **C. Marques:** None. **M.E. Ward:** None. **B.J. Wainger:** F. Consulting Fees (e.g., advisory boards); Quralis, Apic Bio, Q-StateBiosciences.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.01

Topic: C.09.Stroke

Support: AHA Grant

Title: Input specific intrinsic excitability differences in thalamocortical cells after cerebellar stroke

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Abstract: There are approximately 20,000 cerebellar stroke (CbS) cases annually in the United States. Symptoms in these patients can range from ataxia to impaired cognitive function. Stroke therapies are extremely limited and mechanistic studies in animal models at molecular, cellular and systems levels after CbS, are needed to advance the development of better treatments. The cerebellum (Cb) communicates with the rest of the brain via the cerebello-thalamo-cortical pathway. Damage starting at the Cb might carry altered signaling through the pathway via the ventrolateral (VL) thalamus, a major target of cerebellar (Cbr) efferents. Interestingly, evidence has shown that damage to thalamic afferents (cortical or spinal) can lead to increased excitability in the thalamus. Our goal is to investigate if intrinsic excitability of thalamocortical cells (TCCs) would be significantly altered after CbS. To investigate this, TCCs with Cbr afferents, we injected AAV-hSyn-Chief-mRuby in the cerebellar nuclei (CbN) and in SNr nuclei of naïve mice, 3 weeks prior to performing whole-cell slice electrophysiology in the VL thalamus (contralateral to viral injection). A dual step protocol in current clamp was run in naïve and CbS animals. CbS were induced with photo-thrombosis in the artery that supplies the cerebellar cortex 7 days prior to recordings and 2 weeks after viral injections in CbN. Cbr fibers were opto-genetically stimulated with 470nM LED illumination to test for a synaptic response in the analyzed cell. TCCs with cerebellar afferents (Cb-thalamic, n=13) showed significantly higher tonic (2.4 fold) and rebound firing (2.14 fold) rates than TCCs with no Cbr afferents (n=16). Thalamic cells that showed no synaptic response were suspected to receive input from the basal ganglia, specifically from SNr. To corroborate this, tonic and rebound firing rates of TCCs with SNr input (SNr-thalamic) matched the non-responding rates from experiments with Cbr afferent stimulation. We next assessed changes in intrinsic excitability in TCCs following CbS. No significant changes in tonic or rebound firing were observed in Cb-thalamic cells, however, SNr-thalamic cells showed a significant increase in rebound firing compared to control. Our data suggest that TCCs in the VL with Cbr or SNr inputs exhibit distinct excitability properties. Cb-thalamic neurons exhibit high tonic and rebound excitability that is not altered by CbS. The increased excitability in SNr-thalamic neurons is surprising, but perhaps suggests global maladaptive changes in the VL. Future studies will investigate molecular underpinnings in TCCs heterogeneity and stroke-induced excitability changes.

Disclosures: M. Moreno Garcia: None. **N. Quillinan:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.02

Topic: C.09.Stroke

Support: Ability Central Grant

Title: Infratentorial contributions to post-stroke aphasia: insights from transcranial direct current stimulation and diffusion tensor imaging

Authors: *Z. ZHENG[1,2], K. WANG[2], S. LEE[1], J. WANG[1], M. HOWARD[1], E. ROSARIO[1], C. SCHNAKERS[1];
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Abstract: Transcranial direct current stimulation (tDCS) has emerged as a popular adjunct therapy to traditional speech and language therapy (SLT) in post-stroke aphasics to facilitate language recovery. While promising, studies investigating the efficacy of tDCS have yielded variable responses, likely, in part, due to the substantial stroke heterogeneity, especially in lesion location and size. Thus, determining the most optimal stimulation site remains difficult. Here, we aim to evaluate the efficacy of tDCS applied over two different sites, left inferior frontal gyrus (Broca's area) and right posterolateral cerebellum (a novel target), with sham tDCS as the control. Because variable and extensive cortical lesions introduce major methodological limitations that hinder automation and feasibility in a clinical environment, we additionally aim to identify microstructural correlates of post-stroke aphasia that are relatively spared from macrostructural damage. Following a double-blind controlled design, patients received either anodal frontal tDCS (f-tDCS; n=17), anodal cerebellar tDCS (c-tDCS; n=12), or sham stimulation (n=18), concurrent with 20 minutes of SLT over five consecutive days. Behavioral and diffusion-weighted data (64 directions) were collected within one week before and after intervention. The Western Aphasia Battery-Revised (WAB-R), which consisted of four subscales addressing spontaneous speech, name and word finding, repetition, and auditory verbal comprehension, was used to assess aphasia severity (aphasia quotient [AQ]). Mixed ANOVA was used to compare the efficacy of f-tDCS, c-tDCS, and sham. The average fractional anisotropy (FA) and mean diffusivity (MD) values were extracted from selected white matter regions from the JHU White Matter Atlas that were macrostructurally intact across all patients. Correlations were performed between WAB-R scores and diffusion metrics at baseline across all participants. After controlling for age and time since injury, we found the greatest improvements in the f-tDCS group, followed by c-tDCS, and then sham. Moreover, f-tDCS improved spontaneous speech and c-tDCS improved repetition scores in non-fluent patients, while sham did not. Furthermore, we identified significant correlations between various WAB-R scores and white matter integrity in predominantly brainstem and cerebellar tracts, especially the cerebral peduncle and middle cerebellar peduncle. These findings highlight the therapeutic potential of different tDCS targets for post-stroke aphasia and suggest that the integrity of infratentorial white matter tracts may serve as novel biomarkers of aphasia severity.

Disclosures: Z. Zheng: None. **K. Wang:** None. **S. Lee:** None. **J. Wang:** None. **M. Howard:** None. **E. Rosario:** None. **C. Schnakers:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.03

Topic: C.09.Stroke

Support: NIH Grant R01NS093057

Title: Assessment of motor deficits after experimental stroke using staircase reaching test

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Abstract: Keywords: Stroke recovery, side-specific deficit, motor skill, reaching, paw use, behavior test, staircase test
Background: Assessment of rodent neurological functions after experimental stroke has translational applications, as accurate and objective measurements of functional recovery are crucial for evaluating the effectiveness of various treatment strategies. Some of the existing behavior tests assess only motor coordination and general locomotor activity and lack side-specific motor impairments that occur after unilateral lesion such as focal ischemic stroke. Furthermore, effective behavior tests that can evaluate neurological deficits long-term are necessary for focal ischemic stroke models, as these mice recover quickly.
Methods: Adult male C57BL/6J mice (12-14 weeks of age) were subjected to permanent distal middle cerebral artery occlusion (dMCAO, n=10). Naïve mice (n=5) were prepared as no stroke control. Staircase reaching tests were performed using staircase chambers from Lafayette Instruments (30-min sessions). Mice were subjected to a 14-day training period where they were kept on a 21-hour food deprivation regime. The number of steps reached and pellets grasped/eaten were evaluated at pre-stroke baseline and at post-stroke days (PD) 7, 8, 14, and 15. Mice were maintained at 85%–90% of their original body weight. Brains were collected at post-stroke day (PD) 16 and sections were processed for immunostaining using antibodies targeting neurons (MAP2) and/or activated microglia/macrophage (CD68).
Results: After a 14-day training period, mice reached a stable baseline for both reaching and pellets consumed. At PD7 and PD8, stroked mice performed only 88% and 89% of their left and right maximum step reached when compared to pre-stroke baseline, respectively. At PD14 and PD15, stroked mice exhibited a further decline in performance and only reached 65% of their baseline performance on the affected side (right limb) while reaching 92% of their baseline performance on the non-affected side (left limb).
Conclusions: Our results show that the staircase reaching test can detect side-specific motor

deficits at up to post-stroke day15 in a dMCAO model. Ongoing studies are evaluating long-term deficits up to one month post-stroke and assessing the effects of optogenetic circuit stimulations on grasping behavior using staircase test.

Disclosures: A. Kim: None. **Z. Cao:** None. **G.K. Steinberg:** None. **T.C. Chiang:** None. **M.Y. Cheng:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.04

Topic: C.09.Stroke

Support: NIH NINDS 5R01NS112424

Title: Changes in neural population dynamics during stroke recovery in non-human primates

Authors: *H. CHOI[1], P. KHANNA[1], L. NOVIK[2], J. ROBERTS[2], R. J. MORECRAFT[3], K. GANGULY[1];
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Abstract: Stroke is a leading cause of serious long-term motor disability; while there have been significant advances in rehabilitation, a large number of stroke survivors are still left with long-term disability due to upper limb dysfunction. The goal of this study was to fully delineate how neural population dynamics change with motor recovery. Here we specifically focused on spiking activity and local field potentials (LFP). Population spiking activity and LFP have been found to be predictors of motor recovery from stroke in rodents, but this is less clear in non-human primates (NHP) performing a dexterous motor task. Understanding this relationship can help drive the development of novel clinical therapies. In this study, we tracked spiking activity and LFPs in a NHP cortical stroke model along with motor behavior during long-term recovery. During the hand movement task, we recorded neural signals and analyzed how they change with recovery. Spike population dynamics, low-frequency oscillations (LFOs), mu, and beta band powers were calculated. In general, LFOs are known to be linked to movement execution, and mu and beta band powers are known to be involved in movement preparation, e.g. show event-related desynchronization (ERD) at movement onset. We found that movement-related population spiking was increased with the recovery of motor function. LFOs also appeared to follow this population spiking metric. In contrast, mu and beta band power appeared to be most linked to processing prior to movement onset and had a negative correlation with population spiking activity. Our results thus provide additional evidence that changes in spiking and LFP coupling are present with dexterous motor recovery, in general, and in NHPs, in specific. These

findings also suggest precisely how neural dynamics correlate with motor recovery. In addition to providing possible clinical biomarkers to track motor recovery, they also suggest targets for neuromodulation to improve motor function after stroke.

Disclosures: H. Choi: None. **P. Khanna:** None. **L. Novik:** None. **J. Roberts:** None. **R.J. Morecraft:** None. **K. Ganguly:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.05

Topic: C.09.Stroke

Support: CalciGenix, LLC

Title: Intrinsic plasticity following in vitro ischemia in the adult rat hippocampus

Authors: *B. NATWORA[1], M. MASSMAN[1], I. MORLEY[1], J. R. MOYER, JR.[1,2];
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Abstract: Stroke is the fifth leading cause of death in the United States, killing nearly 150,000 of the 860,000 Americans who die of cardiovascular disease each year. Approximately 87% of those cases are the result of an ischemic stroke (Virani, *Circulation*, 2020), characterized by the occlusion of an artery that supplies oxygen-rich blood to the brain. Neurons within the territory of the blocked vessel exhibit increased excitability due to the accumulation of extracellular glutamate, causing an overload of Ca^{2+} and ultimately delayed neuronal death (Choi, *Neurosci Lett*, 1985). The present study tested the hypothesis that exposure of brain slices to *in vitro* ischemia alters neuronal membrane properties, excitability and structural integrity of hippocampal CA1 pyramidal neurons. Brain slices prepared from adult Fisher 344 rats were subjected to 5 min of oxygen-glucose deprivation (OGD) after which slices were placed back in oxygenated aCSF (control slices remained in oxygenated aCSF). Within a subset of control and OGD-treated slices from each rat, cell death was assayed by trypan blue exclusion (Detert, *PLoS One*, 2013). For the remaining slices, whole-cell recordings were obtained from CA1 neurons to study basic membrane properties and intrinsic excitability. Preliminary data suggest that neurons from OGD-treated slices exhibit significantly reduced intrinsic excitability, as evidenced by a decrease in the number of action potentials following a stepwise current injection as well as a reduced neuronal input resistance (R_N). Neurons from OGD-treated slices also displayed a reduction in spike threshold and hyperpolarization-activated current (I_h) relative to those from control-treated slices. Interestingly, confocal images from biocytin-filled neurons suggest that some neurons from OGD-treated slices displayed dendritic beading, a morphological change commonly seen in neurons following a stroke. Additionally, OGD-treated slices had a

significantly greater number of trypan blue-labeled neurons compared to control-treated slices, confirming the effectiveness of our OGD protocol. While preliminary, these results suggest that *in vitro* ischemia leads to changes in neuronal membrane excitability, which may impact susceptibility to cell death and recovery of function. Understanding the nature of these changes may also shed light on mechanisms that underlie intrinsic plasticity within hippocampal CA1 pyramidal neurons and provide an avenue to study neurotherapeutics that could reverse the damage inflicted by ischemic stroke.
Funding: CalciGenix, LLC

Disclosures: B. Natwora: None. **M. Massman:** None. **J.R. Moyer, Jr.:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.06

Topic: C.09.Stroke

Support: Mäxi Foundation
Swiss 3R Competence Centre (3RCC)
Swiss National Foundation (SNF)

Title: Deep-learning based behavioral profiling of rodent stroke recovery

Authors: R. WEBER[1], G. MULDERS[1], J. KAISER[3], C. TACKENBERG[2], ***R. RUST**[1]; [1]Inst. for Regenerative Med., Univ. of Zurich, Schlieren, Switzerland; [2]Inst. for Regenerative Med., Univ. of Zurich, Schlieren, ZH, Switzerland; [3]Novago, Schlieren, Switzerland

Abstract: Patients suffering from ischemic strokes have limited therapeutic options and are often left with considerable disabilities. To promote neurological recovery, a variety of experimental strategies have evolved to enhance regeneration of damaged neuronal circuits. These preclinical studies heavily rely on behavioral improvements to evaluate the treatment's efficacy. Although behavior evaluation is considered important, tests in rodents are still often poorly reproducible and often not suitable to unravel the complex behavior after stroke. Here, we present a comprehensive 3D gait analysis of mice after focal cerebral ischemia based on the new deep-learning based software that requires only basic behavioral equipment. We developed an experimental set-up to perform high-precision tracking up to 10 body parts of several mouse strains. This approach enables to identify new biologically relevant differences in locomotor profiles following stroke across a time course of three weeks. We further directly compare the performance of deep-learning based motion tracking to conventional assessments including horizontal rung walk, cylinder test and single-pallet grasping with regard to sensitivity, accuracy and time consumption. Overall, we conclude that deep learning based motion tracking with

comprehensive post-analysis provides accurate and sensitive data to describe complex recovery of rodents following a stroke. The experimental set-up and analysis may also be applied to a range of other neurological injuries that affect motor function.

Disclosures: R. Weber: None. **G. Mulders:** None. **J. Kaiser:** None. **C. Tackenberg:** None. **R. Rust:** None.

Poster

P330. Assessment of Neuronal Population Function After Stroke

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P330.07

Topic: C.08. Ischemia

Support: NIH Grant K12HD07394
NSF Grant EEC-102872
NIH R01NS102886
VA Merit Award I01BX00333
VA Research Career Scientist award IK6BX00460

Title: Local field potentials identify features of hippocampal activity and cortico-hippocampal communication which are impacted by stroke and environmental enrichment therapy

Authors: *Z. IP[1], G. RABILLER[3], J.-W. HE[3], S. CHAVAN[1], Y. NISHIJIMA[4], Y. AKAMATSU[3], J. LIU[5], A. YAZDAN-SHAHMORAD[2];
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Abstract: Stroke is a leading cause of disability with more than 15 million people affected by stroke every year. Impaired cognition is a common consequence following stroke, though how middle cerebral artery (MCA) stroke affects the neural activity of the hippocampus, an area critical for memory is not well understood. Environmental enrichment (EE) is a therapy that has consistently been shown to improve cognition following stroke, yet the electrophysiology that underlies this improvement is not well understood. To gain a deeper understanding of EE in the context of stroke we induced ischemic stroke in the rats. After recovery and subsequent EE therapy, we used urethane anesthesia and recorded local field potentials simultaneously from sensorimotor cortex & hippocampus. We analyzed the stability of sleep like brain states in the hippocampus, characteristics of sharp wave associated ripples (SPW-Rs) and the current source density surrounding SPW-Rs, and phase amplitude coupling within the hippocampus and between the cortex and the hippocampus. Our results show that MCA stroke impacts the electrophysiology in the hippocampus, disrupting the stability of brain states, characteristics of

SPW-Rs, and PAC both within the hippocampus and between hippocampus and cortex. Subsequent EE therapy mitigated changes to SPW-R characteristics, however, interestingly, EE further disrupted brain state and coupling both in controls and following stroke. These results show that despite having no direct lesion to the hippocampus, stroke strongly affects the electrophysiology within the hippocampus, and communication between the hippocampus and the cortex. These results provide a valuable set of neural features to take into consideration when developing therapeutic strategies for stroke.

Disclosures: Z. Ip: None. **G. Rabiller:** None. **J. He:** None. **S. Chavan:** None. **Y. Nishijima:** None. **Y. Akamatsu:** None. **J. Liu:** None. **A. Yazdan-Shahmorad:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.01

Topic: C.10. Brain Injury and Trauma

Support: NIH 5R01NS104282-03

Title: CLIP antagonism improves neurobehavioral outcomes after Traumatic Brain Injury

Authors: *L. VENKATASAMY[1], S. BEEVERS[1], J. IANNUCCI[1], V. ARISMENDI[1], K. MONTGOMERY[1], K. NEWELL ROGERS[2], L. A. SHAPIRO[1];
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Abstract: Background:Traumatic Brain Injury (TBI) can cause permanent damage to the brain, life-long disability and is animportant worldwide health problem. Millions of people each year suffer a TBI and post-traumaticsymptoms are often highly debilitating. In addition to neurodegeneration, those who suffered a TBI candevelop cognitive, affective and behavioral impairments, and are at a higher risk of developing addiction,alcoholism, post-traumatic epilepsy and Alzheimer's disease. However, the underlying mechanisms arenot fully understood, and therapeutic options are limited. Innate and adaptive immune responses playimportant roles in the inflammatory response following a TBI. The full-length CD74 molecule contributesto several mechanisms of the innate immune response, and MHC II-associated invariant peptide (CLIP), acleavage product of CD74, is involved in the transition from an innate to an adaptive immune response.Depletion of CD74, or CLIP antagonism, was previously shown to prevent the secondaryneurodegeneration in a fluid percussion injury (FPI) model of TBI. However, the role of CLIP antagonismin improving functional post-traumatic outcomes has not been previously examined.Hypothesis:CLIP antagonism after TBI will improve neurobehavioral impairment.Methods:The Fluid Percussion Injury (FPI) model was induced in male and female

C57BL/6 mice, as previouslydescribed. Mice were injected (1 mg/kg, i.p.) with our CLIP antagonist peptide (CAP) at 30 mins after FPIor sham FPI. Vehicle animals (VEH) received DMSO in place of CAP. We performed the followingneurobehavioral tests beginning 32 days after FPI: Object location test (OLT), Novel object recognitiontest (NORT) and pattern separation test (PST).Results:We observed behavioral deficits after FPI and observed improvements in behavioral outcomes in thosemice treated with CAP. In OLT, a significant number of the FPI+CAP mice visited the novel place objectfirst, whereas FPI+VEH mice showed no significant preference between the novel and familiar placeobject. In NORT, FPI+CAP mice spent significantly more time exploring the novel object than the familiarobject, whereas FPI+VEH mice showed no preference for the novel object compared to the familiarobject. These results suggest an impaired object recognition memory after TBI that is rescued byantagonizing CLIP with CAP. Follow-up studies are planned to test the immune response profiles andtheir influence on these behavioral outcomes.

Disclosures: L. Venkatasamy: None. **S. Beevers:** None. **J. Iannucci:** None. **V. Arismendi:** None. **K. Montgomery:** None. **K. Newell Rogers:** None. **L.A. Shapiro:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.02

Topic: C.10. Brain Injury and Trauma

Support: NINDS Grant 1R01NS106907

Title: Intranasal Treatment of Human MSC-derived EVs Enriched with Microglia Modulating miRNAs after TBI Prevents Cognitive and Mood Dysfunction with Inhibition of NLRP3 Inflammasomes

Authors: *M. KODALI[1], R. REGER[1], M. BOJANA[1], L. N. MADHU[1], R. UPADHYA[1], J. GONZALEZ[1], S. ATTALURI[1], B. SHUAI[1], D. GITAI[2], J. M. CHOI[3,4], S. Y. JUNG[4], A. K. SHETTY[1];
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Abstract: Cognitive and mood impairments allied with chronic neuroinflammation and diminished neurogenesis are among the most conspicuous features in the chronic phase after traumatic brain injury (TBI). One approach to mitigating these chronic alterations is administering extracellular vesicles (EVs) secreted by human mesenchymal stem cells (hMSCs)

due to their well-known antiinflammatory, neuroprotective, and neurogenic properties. Our previous study showed that intravenous delivery of hMSC-EVs could reduce acute neuroinflammation and improve cognitive function in the early post-injury period (Kim et al., PNAS, 2016). This study investigated the dose-response effects of a single intranasal (IN) administration of hMSC-EVs enriched with microglia modulating microRNAs (miRs) early after a moderate TBI. The antiinflammatory property of EVs employed in the study was validated through an LPS-stimulated in vitro macrophage assay. A small RNA-seq study also confirmed that the EVs were enriched with miRs capable of modulating microglia and inhibiting NLRP3 inflammasomes. Nine-week-old male mice were subjected to unilateral controlled cortical impact injury (impactor diameter-3mm, velocity-5m/s, dwell time-300ms, and injury depth-0.8 mm). Ninety minutes later, mice received an IN administration of EVs (6.4, 12.8, or 25.6 billion doses) or the vehicle (TBI-VEH). Three months later, a series of behavioral tests examined cognitive and mood function. TBI-VEH mice displayed recognition memory problems, pattern separation deficits, anhedonia, and anxiety. However, TBI mice that received higher doses of EVs (12.8 and 25.6 billion) displayed preserved cognitive, memory, and mood function. These functional benefits were linked with reduced astrocyte hypertrophy and microglial density, an enhanced ratio of noninflammatory M2 microglia vis-à-vis the proinflammatory M1 microglia in the hippocampus, and somatosensory cortex, ipsilateral (IL) to injury. Remarkably, EV treatment also inhibited the activation of NLRP3 inflammasomes, with downregulation of NF-kB, caspase-1, IL-1β, and IL-18 in the somatosensory cortex. Proteomic analysis revealed that hMSC-EV treatment inhibited the inflammatory proteome in the hippocampus IL to the injury. TBI-EV mice also showed a higher level of neurogenesis and reduced synapse loss in the hippocampus IL to the injury. Thus, IN hMSC-EV treatment after a moderate TBI is efficacious for preventing long-term cognitive and mood dysfunction. Inhibition of NLRP3 inflammasomes and inflammatory proteome and preservation of neurogenesis in the hippocampus likely underlie such beneficial effects.

Disclosures: M. Kodali: None. **R. Reger:** None. **M. Bojana:** None. **L.N. Madhu:** None. **R. Upadhya:** None. **J. Gonzalez:** None. **S. Attaluri:** None. **B. Shuai:** None. **D. Gitai:** None. **J.M. Choi:** None. **S.Y. Jung:** None. **A.K. Shetty:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.03

Topic: C.10. Brain Injury and Trauma

Support: OAA VA Advanced Fellowship in Polytrauma/Traumatic Brain Injury Rehabilitation; Department of Veterans Affairs

Paul Kalmanovitz Central Nervous System Repair Research Program; Department of Veterans Affairs

Title: Anti-Nogo-A antibody therapy improves functional recovery from traumatic brain injury in rats

Authors: *B. POWERS[1], S.-Y. TSAI[1], S. TON[1], R. FARRER[1], G. KARTJE[1,2];
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Abstract: Traumatic brain injury (TBI) results in debilitating outcomes including deficits in sensory and motor function, and recovery is often slow and incomplete. In addition to brain tissue damage from the immediate trauma, head injuries may have long-term effects that increase the risk for development of mood and dementia disorders. Unfortunately, there are no effective pharmacological treatments for recovery from TBI, but research suggests great potential demonstrated by anti-Nogo-A antibody therapy. This therapy is designed to neutralize Nogo-A, an endogenous protein that inhibits neuronal plasticity. In the current study, we modeled TBI in adult male, Long Evans rats, using the controlled cortical impact (CCI) method, which results in focal brain damage and induces motor deficits very similar to the effects of TBI in humans. We hypothesized that treatment with anti-Nogo-A neutralizing antibodies following TBI would result in disinhibited axonal and dendritic growth, the establishment of new compensatory synaptic connections, and improvement in functional outcome. Prior to TBI, subjects were trained to a high level of proficiency on the skilled forelimb reaching task, and we assessed baseline performance on the skilled ladder rung walking task as well as two ethological measures of anxiety in rodents, the elevated zero maze and the light/dark apparatus. Subjects were given a TBI, and one week later were randomly divided into 3 groups: TBI only (n=7), TBI + anti-Nogo-A antibody (n=8), and TBI + control antibody (n=8). Testing on the skilled forelimb reaching task and skilled ladder rung walking task resumed 3 days after TBI and continued until 8 weeks after TBI. The tests for anxiety were repeated at 8 weeks after TBI. At the conclusion of behavioral testing, subjects received an injection of the neuronal tracer biotinylated dextran amine (BDA) into the motor cortex contralateral to the injury. Our behavioral results indicate significant improvement in motor functional recovery in rats that received anti-Nogo-A antibody therapy. Results for measures of anxiety as well as anatomical evidence of new neuronal pathways are still under investigation. This study indicates that anti-Nogo-A antibody therapy may be a promising treatment to improve functional recovery in humans who have suffered a TBI.

Disclosures: B. Powers: None. **S. Tsai:** None. **S. Ton:** None. **R. Farrer:** None. **G. Kartje:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.04

Topic: C.10. Brain Injury and Trauma

Support: (NIA) R01-AG-051902
(NINDS) P30-NS-045758

Title: Amplified cognitive deficits, gliosis, and interferon-associated neuroinflammation in aged mice after diffuse brain injury

Authors: *L. WANGLER[1], C. BRAY[1], J. PACKER[1], S. M. O'NEIL[2], M. OUVINA[3], M. WITZEL[3], K. BAETZ[3], E. HANS[3], J. GODBOUT[1];
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Abstract: Traumatic brain injury (TBI) can lead to significant neuropsychiatric complications and neurodegenerative pathologies. Moreover, aged individuals are particularly susceptible to fall-related diffuse brain injuries and subsequently exhibit worse functional impairment. Although the vulnerability of older individuals to complications following brain injury is well-recognized, the mechanisms by which this occur are unclear. There is evidence of increased neuroinflammation and microglial priming in aging, and these factors may underlie the increased neuropathology and impaired functional recovery in aged individuals after TBI. Therefore, we aimed to determine the degree to which neuroinflammation, neuropathology, and cognitive decline were increased following TBI as a function of age. In these experiments, adult and aged C57Bl/6 mice received a TBI using a midline fluid percussive injury (mFPI). Several cognitive and biochemical parameters were assessed at subacute (7 days post injury, dpi) and chronic (30 dpi) time-points. Aged mice had increased time to right after mFPI compared to adults. While both adult and aged mice showed impairment in Novel Object Recognition (NOR) and Novel Object Location (NOL) memory, Aged-TBI mice showed exacerbated deficits at 7 and 30 dpi. Moreover, histological analysis of microglia and astrocytes showed Aged-TBI mice had augmented cortical gliosis, and mRNA analysis showed an enhanced neuroinflammatory profile with evidence of microglial priming (Clec7a, H2-Eb1, Itgax) at 7 dpi. In addition, cortical mRNA analysis revealed a robust inflammatory signature associated with type 1 interferons in the Aged-TBI mice. For instance, at 7 and 30 dpi there was exacerbated expression of Tmem173, Irf7, and Ifi27l2a in the Aged-TBI mice compared to Adult-TBI. Collectively, these studies indicate that aged mice have enhanced cognitive decline, neuroinflammation, and neuropathology after TBI that is associated with pronounced type 1 interferon signaling.

Disclosures: L. Wangler: None. **C. Bray:** None. **J. Packer:** None. **M. Ouvina:** None. **M. Witzel:** None. **K. Baetz:** None. **E. Hans:** None. **J. Godbout:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.05

Topic: C.10. Brain Injury and Trauma

Support: Children's Hospital of Pittsburgh, UPMC Health System
NIH R01-NS106925
NIH R21-NS115440
PA Department of Health-4100077083

Title: A retinoic acid dose response study: evaluating therapeutic effects on hippocampal synaptic protein expression and cognitive function after traumatic brain injury.

Authors: *S. SVIRSKY[1], J. HENCHIR[1], Y. LI[1], M. PARRY[1], E. HOLETS[1], S. W. CARLSON[1], C. DIXON[1,2];
[1]Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; [2]Pittsburgh VA, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) is known to impair synaptic function, and subsequently contributes to observed cognitive deficits. Retinoic acid (RA), a metabolite of Vitamin A, has been identified as a potential pharmacotherapeutic for experimental ischemic injury and other neurological disorders, partially due to increased expression of synaptic signaling and plasticity proteins. This study aims to understand the effect of various doses of RA on improving hippocampal synaptic protein expression and learning and memory behaviors after TBI. Adult, male Sprague Dawley rats (275-300g, 9-10 per group) received either controlled cortical impact (CCI, 2.5mm deformation, 4m/s) or control/sham surgery. Animals received daily intraperitoneal injection of 0.5, 1, 5, or 10 mg/kg body weight of RA or DMSO-vehicle for 2-weeks. Animals underwent Morris Water Maze (MWM) testing days 9-14 post-injury. Neurogranin (Ng), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), post-synaptic density protein 95 (PSD-95) and synaptophysin expression was measured in hippocampal synaptosomal lysates by western blot at 2-weeks post-injury. A repeated measures ANOVA showed an overall group effect in MWM latency to reach submerged platform (p=0.001). *Post-hoc* analysis revealed a significant injury effect between Sham and CCI vehicle groups (p=0.004). No significant differences between Sham-vehicle and CCI-0.5, 1 and 5mg dose groups were observed. A one-way ANOVA of synaptosomal Ng and CaMKII expression showed an overall group effect (p=0.002, p&lt0.0001). *Post-hoc* analysis showed a significant injury effect between Sham and CCI-vehicle groups (p=0.0025, p=0.0027), but no significant difference between Sham-vehicle and CCI-1 and 5mg doses. Furthermore CCI-1mg dose showed a trending increase in Ng expression from the CCI-vehicle group (p=0.0583). No significant group effect was observed in PSD-95 and synaptophysin expression. In conclusion, RA had a modestly positive effect on both behavior and hippocampal synaptic protein expression, with doses demonstrating similar effects across behavioral and biochemical outcomes. Further work is needed to investigate mechanisms of RA signaling in TBI-induced cognitive dysfunction, given the pleiotropic effects of RA.

Disclosures: S. Svirsky: None. **J. Henchir:** None. **Y. Li:** None. **M. Parry:** None. **E. Holets:** None. **S.W. Carlson:** None. **C. Dixon:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.06

Topic: C.10. Brain Injury and Trauma

Support: NIH RO1 NS1168383

Title: The effects of 7,8-Dihydroxyflavone and exercise on brain atrophy, functional connectivity, and behavior following mild to moderate TBI

Authors: *G. SMITH[1,2], A. PAYDAR[1,2], L. ZHE YING[3], G. KRISHNA[3], N. HARRIS[1,2], F. GOMEZ-PINILLA[1,2,3];
[1]Neurosurg., UCLA David Geffen Sch. of Med., Los Angeles, CA; [2]UCLA BIRC, Los Angeles, CA; [3]Dept. Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: 7,8-Dihydroxyflavone (DHF) an agonist of the Brain-derived neurotrophic factor (BDNF)/tropomyosin-related kinase B (TrkB) receptor has been shown to be neuroprotective. The ability of DHF alone and in combination with exercise to normalize brain structure and function following mild to moderate traumatic brain injury (TBI) was assessed using adult Sprague Dawley rats (n=55) in a fluid percussion injury (FPI) model. Some of the long-term effects of TBI are chronically altered MRI based metrics such as increased mean diffusivity (MD) and decreased fractional anisotropy (FA), as well as increases in local tissue atrophy within the brain. These indicators of micro and macro-structural damage in the brain following TBI occur commensurate with a loss of function connectivity that may underlie persistent deficits in multiple behavioral processes. We tested the effects of DHF, either administered alone or in combination with exercise starting at post injury day (PID) 1 to PID 14, to normalize the degree of structural changes after TBI that will result in improved functional connectivity. This improvement in connectivity will help improve cognitive function and reduce anxiety-like behaviors.
Following injury rats were assessed for anxiety-like behaviors using open field and elevated plus maze, and cognitive function was measured using a Barnes maze which the rats had been previously trained on. Functional and structural MRI data were also acquired at 2 wks post-injury using a standard resting state acquisition protocol with dexmedetomidine sedation followed by an anatomical scan to assess tissue atrophy.
Preliminary analysis of these data indicates that administration of DHF either alone or in combination with exercise help normalize local tissue atrophy resulting in improved functional connectivity in the rat brain following FPI.

Disclosures: G. Smith: None. **A. Paydar:** None. **L. Zhe Ying:** None. **G. Krishna:** None. **N. Harris:** None. **F. Gomez-Pinilla:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.07

Topic: C.10. Brain Injury and Trauma

Support: DOD/USAMRAAADC W81XWH-18-1-0388

Title: Epigenetics, neuroinflammation:Possible targets for therapy following traumatic brain injury (TBI)

Authors: *D. P. CROCKETT[1], V. L. DIBONA[2], H. CAIOLA[1], E. C. KELLY-CASTRO[1], M. VOGLEWEDE[1], N. BADADE[1], N. MALLALIEU[1], H. ZHANG[1];
[1]Neurosci. and Cell Biol., Rutgers-Robert Wod Johnson Med. Sch., Piscataway, NJ; [2]Pathology, Rutgers-Robert Wood Jonsnon Med. Sch., New Brunswick, NJ

Abstract: Each year, traumatic injury to the brain or to the spinal cord will afflict millions of people around the world. At present, there is no effective cure. An effective treatment will have to addresses a wide range of issues. One factor that will need to be addressed is the ongoing neuroinflammation triggered by injury. Prolonged inflammation can lead to continued loss of neurons and oligodendrocytes and consequently loss of function. Major players in the inflammatory response include the microglia and the astrocytes. Both cell types undergo major morphological changes as part of their response to injury which requires transcriptional regulation. Microglia are highly ramified at “rest” but change into rounded phagocytic cells following injury. The mechanism associated with this activation and morphological change is not completely understood. Likewise, the injury response of astrocytes includes numerous biochemical, morphological, metabolic, and physiological remodeling (Escartin et al 2021). We have recently demonstrated that the polarity protein Par1b/MARK2 may be instrumental in the inflammatory response to TBI (DiBona et al 2019). The loss of Par1b/MARK2 facilitates the microglial inflammatory response in both primary microglia *in vitro* and following traumatic brain injury (TBI) in mice. Par1b/MARK2 is a serine/thionine kinase and is a member of Par1/MARK (microtubule affinity regulating kinase) family of polarity proteins. One possible pathway that Par1b/MARK2 may influence microglial activation may include the class IIa histone deacetylase, HDAC7. Previously, HDAC7 has been shown to play an important role in macrophage immune responses (Shakespeare et al 2013 and Gupta et al 2020). Par1b/MARK2 has been shown to regulate cellular localization, and function of the class IIa HDACs through N-terminal phosphorylation (Dequiedt et al 2006). Interestingly, we have observed an increase in HDAC7 immunostaining in both activated microglia and reactive GFAP+ astrocytes within cortex following traumatic injury. We are currently examining the link between Par1b/MARK2

and the class IIa HDACs following TBI in both juvenile and adult mice and are examining other epigenetic factors that may play an important role in the inflammatory response to injury.

Disclosures: D.P. Crockett: None. **V.L. DiBona:** None. **H. Caiola:** None. **E.C. Kelly-Castro:** None. **M. Voglewede:** None. **N. Badade:** None. **N. Mallalieu:** None. **H. Zhang:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.08

Topic: C.10. Brain Injury and Trauma

Support: NIH grant HD001097
CCHMC Procter Scholar Award

Title: Brief oxygen exposure following blunt head trauma improves survival and reduces pathologic signs of traumatic optic neuropathy with variable, time-dependent ER stress responses in the retina.

Authors: *S. M. HETZER[1], N. K. EVANSON[2];
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Abstract: Visual deficits after traumatic brain injury (TBI) develop in 50-60% of patients with 5% occurring because of injury to the optic nerve (ON). Symptoms of this traumatic optic neuropathy vary and can worsen for up to 35 years after TBI. Current literature predominantly follows adult subjects, despite a significant incidence of visual deficits in 20% of pediatric TBIs. We previously reported that adolescent mice experience higher mortality and worse histological outcomes following blunt head injury, compared to adult mice. We also found that Endoplasmic Reticulum (ER) stress and ER-mediated reactive oxygen species (ROS) proteins are elevated following ON injury in the retina. Because elevated adolescent mortality appeared to be due to post-impact apnea, we hypothesized that a brief exposure to oxygen (O_2) after injury would improve survival but that increased O_2 would be toxic to retinal cells by increasing ER stress and ROS production. To test this, we utilized a single weight-drop injury in adolescent male mice who were either exposed to 100% O_2 by hood until normal breathing returned (~3 min) or allowed to recover in room air. We followed mice 7- and 30-days post injury (DPI) and examined (a) behavioral measures of visual function, (b) histological measures in retina and brain tissue for retinal ganglion cell loss, axonal degeneration, and glial reactivity, and (c) molecular changes in the retina involving markers of ROS and ER stress via western blot. We found that O_2 reduces adolescent mortality by 40%, improves post-injury visual acuity, and reduces axonal degeneration and gliosis in central ON projection targets 7- and 30-DPI. ER

stress marker expression was altered in injured mice overall, and mice given O_2 appear to utilize different ER-stress pathways in a time dependent manner, compared to other experimental groups. O_2 exposed mice had increased expression of IRE-1α pathway markers 7 DPI and increased PERK pathway expression 30 DPI. Room air exposed mice showed the opposite pattern of expression at 7 and 30 DPI. O_2 exposed mice did not have significantly elevated ER-mediated apoptosis markers (i.e., CHOP) 30 DPI, while room air mice did. Finally, brief O_2 exposure reduced ER-mediated ROS production, as seen through significantly reduced PDI and ERO1α expression, compared to room air and uninjured mice. Taken together these results suggest that a single, short exposure to O_2 immediately following head trauma is beneficial to survival and ON injury outcomes, potentially by altering ER stress response pathways. Our results suggest that changing the oxygen/redox balance immediately following TBI has significant effects on ER stress responses to optic nerve trauma.

Disclosures: S.M. Hetzer: None. **N.K. Evanson:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.09

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA R01AG057705 (to G. Enikolopov and H. Benveniste)
T32 training fellowship 5T32GM086287-12 (to Z. Gursky)

Title: Glymphatic transport and drainage to the deep cervical lymph nodes is sustained in ciliopathy with hydrocephalus

Authors: *Z. H. GURSKY[1], Y. XUE[1], B. MONTE[1], S. CONSTANTINOU[1], X. LIU[1], S. KOUNDAL[1], H. LEE[1], A. NEMAJEROVA[2], T. MICHURINA[3], K. MELLANSON[3], K.-I. TAKEMARU[4], G. ENIKOLOPOV[3], N. PEUNOVA[3], H. BENVENISTE[1];
[1]Dept. of Anesthesiol., Yale Sch. of Med., New Haven, CT; [2]Dept. of Pathology, [3]Dept. of Anesthesiol., [4]Dept. of Pharmacol. Sci., Renaissance Sch. of Med. at Stony Brook Univ., Stony Brook, NY

Abstract: Multiciliated cells (MCC) serve many functions in the developing and mature brain, lining the ventricles of the brain, the airways, and the ducts of reproductive organs. The contribution of MCC to CSF motion and central nervous system (CNS) fluid homeostasis is poorly understood. In two separate experiments, we utilized two adult mouse models of ciliopathy with hydrocephalus — knockout of global p73 (i.e., missing both TAp73 and DeltaNp73 isoforms) [Yang et al., 2000, *Nature*] or FOXJ1-conditional knockout of CEP164 [Siller et al., 2017, *PLoS Genetics*]— to examine glymphatic system function and waste drainage

patterns from the CNS. Both ciliopathic mouse models presented with the communicating form of hydrocephalus. Brain tissue volume was normal in the FOXJ1-Cre;CEP164$^{fl/fl}$ compared to CEP164$^{fl/fl}$ control mice, but reduced by 25% in p73$^{-/-}$ compared to p73$^{+/+}$. Hippocampal anatomy was substantially altered in p73$^{-/-}$ (but not CEP164) mutant mice, manifested as visible folding of pyramidal cell layers, an 'underdeveloped' lower granular cell layer with an abnormal shape of the dentate gyrus, and atypical expression of mature neuronal marker NeuN. Glymphatic transport at 1 hour after infusion of a Gd-based contrast agent (Gd-DOTA) and quantified using T1 mapping was similar across the FOXJ1-Cre;CEP164$^{fl/fl}$ and CEP164$^{fl/fl}$ groups. However, when correcting for differences in total intracranial volume, glymphatic transport in p73$^{-/-}$ was increased by 20% compared to p73$^{+/+}$ mice. Supporting these observations, there were no significant alterations in AQP4 water channel expression along capillaries or small vessels in the ventral hippocampus of FOXJ1-Cre;CEP164$^{fl/fl}$ mice. However, p73$^{-/-}$ mice exhibited a significant increase in AQP4 polarization around capillaries in the ventral hippocampus. Glymphatic transport function is dependent on egress of interstitial fluid and CSF along meningeal lymphatics and cervical lymphatic vessels. Drainage of Gd-DOTA to the nasal conchae was significantly reduced in both ciliopathic mouse models likely due to impaired lymphatic drainage to the submucosa covering the spiral lamellae, secondary to inflammation caused by purulent sinusitis. Notably, drainage of Gd-DOTA to the deep cervical lymph nodes in the ciliopathic mice strains was within normal ranges. The relationship between intracranial pressure and glymphatic-lymphatic transport in the two ciliopathic mouse strains remains a topic for future study.

Disclosures: Z.H. Gursky: None. **Y. Xue:** None. **B. Monte:** None. **S. Constantinou:** None. **X. Liu:** None. **S. Koundal:** None. **H. Lee:** None. **A. Nemajerova:** None. **T. Michurina:** None. **K. Mellanson:** None. **K. Takemaru:** None. **G. Enikolopov:** None. **N. Peunova:** None. **H. Benveniste:** None.

Poster

P340. Animal Models of Brain Injury: Inflammatory Mechanisms and Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P340.10

Topic: C.01. Brain Wellness and Aging

Title: Unexpected source and function of brain perivascular macrophages in health and diseases.

Authors: *A. DRIEU, J. RUSTENHOVEN, S. STORCK, T. MAMULADZE, Z. PAPADOPOULOS, I. SMIRNOV, J. KIPNIS;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Unexpected source and function of brain perivascular macrophages in health and diseases.

Abstract:
Microglial cells are the resident macrophages of the brain. Perivascular macrophages (PVM) are also myeloid resident cells that are located at the interface between blood, CSF and brain in the perivascular space. Previous studies showed that PVM and microglia are coming from early erythro-myeloid progenitors from the yolk sac, but how they repopulate throughout life remained unknown. We now demonstrate that upon depletion, PVM are provided by the skull bone marrow source. Intrigued by this result, we decided to study the role of PVM in the homeostatic brain. Due to their strategic location and their ability to sample CSF content, we hypothesized that PVM might play a role in CSF flow. Using different fluorescent tracers injected either into the CSF (influx) or the brain parenchyma (efflux) we assessed their spatio-temporal distribution and found that ablation (using clodronate liposomes) or dysfunction (using genetic tools) of PVM alters CSF dynamics and patterns. Interestingly, aging results in altered PVM phenotype. Reversing this phenotype using macrophage specific grown factors, reversed some of aging-associated dysfunctions of CSF flow.In summary, our results identify a new role of PVM in CSF flow and open new avenues for therapeutical applications targeting PVM in neurodegenerative diseases such as Alzheimer's disease.

Disclosures: A. Drieu: None. **J. Rustenhoven:** None. **S. Storck:** None. **T. Mamuladze:** None. **Z. Papadopoulos:** None. **I. Smirnov:** None. **J. Kipnis:** None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.01

Topic: D.03. Somatosensation – Pain

Support: NIH R01NS117484

Title: Downregulation of Dorsal Root Ganglion-Specifically Enriched Long Noncoding RNA (DS-lncRNA) negatively regulated RALY-Triggered Ehmt2 Expression after peripheral nerve injury

Authors: *X. FENG;
Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Downregulation of a Dorsal Root Ganglion-SpecificallyEnriched Long Noncoding RNA is Required for NeuropathicPain by Negatively Regulating RALY-Triggered Ehmt□ExpressionDownregulation of a Dorsal Root Ganglion-SpecificallyEnriched Long Noncoding RNA is Required for NeuropathicPain by Negatively Regulating RALY-Triggered Ehmt□ExpressionDownregulation of a Dorsal Root Ganglion-SpecificallyEnriched Long Noncoding RNA is Required for NeuropathicPain by Negatively Regulating RALY-Triggered

Ehmt□ExpressionDownregulation of Dorsal Root Ganglion-Specifically Enriched Long Noncoding RNA (*DS-lncRNA*) negatively regulated RALY-Triggered Ehmt2 Expression after peripheral nerve injury

Xiaozhou Feng, Zhiqiang Pan, Shibin Du, Kun Wang, Huijuan Hu, Yuan-Xiang TaoDepartment of Anesthesiology, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ07103, USA. Nerve injury-induced changes in gene expression in the dorsal root ganglion (DRG) contribute to neuropathic pain genesis. Recent evidence revealed the dysregulated expression of long non-coding RNAs (lncRNAs) in the damaged nerve, dorsal root ganglion (DRG), and spinal cord dorsal horn following peripheral nerve injury. However, the role of the majority of lncRNAs in neuropathic pain is still elusive. We recently identified a unique and native lncRNA highly expressed in mammalian DRG, named DRG-specifically enriched lncRNA (*DS-lncRNA*). Our pilot data demonstrated that nerve injury-induced downregulated of the *DS-lncRNA* in injured DRG contributed to neuropathic pain genesis. However, the mechanism underlying this phenomenon is still unclear. Here, we show that rescuing *DS-lncRNA* downregulation blocks nerve injury-induced increases in the transcriptional cofactor RALY-triggered DRG *Ehmt2* mRNA and its encoding G9a protein, reverses the G9a-controlled downregulation of opioid receptors and Kcna2 in injured DRG, and attenuates nerve injury-induced pain hypersensitivities. Conversely, *DS-lncRNA* downregulation increases RALY-triggered Ehmt2/G9a expression and correspondingly decreases opioid receptor and Kcna2 expression in DRG, leading to neuropathic pain symptoms in mice in the absence of nerve injury. *DS-lncRNA* contributes to neuropathic pain through participation in negatively regulated RALY-Triggered *Ehmt2*/G9a expression in injured DRG.

This work was supported by NIH R01NS117484.

Disclosures: X. feng: None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01NS117484

Title: Downregulationof DS-lncRNAin DRG contributes to neuropathic pain

Authors: *G. GOVINDARAJALU;
Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Downregulation of DS-lncRNA in DRG contributes to neuropathic pain
Gokulapriya Govindarajalu, Zhiqiang Pan, Shibin Du, Kun Wang, Huijuan Hu, Yuan-Xiang Tao

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Neuropathic pain affects the quality of life of 7-10% of the world's population. Current treatments for this debilitating disorder have limited effectiveness and/or produce severe adverse effects. Understanding the mechanisms by which nerve injury causes pain hypersensitivity may provide a new avenue for neuropathic pain management. Nerve injury-induced alterations in gene expression in the dorsal root ganglion (DRG) are considered to be critical in neuropathic pain genesis. Long non-coding RNAs (lncRNAs) are emerging as potent and multifunctional regulators of gene expression. Here, we reported a unique and native lncRNA highly expressed in mammalian DRG, named DRG-specifically enriched lncRNA (*DS-lncRNA*). *DS-lncRNA* contains a long and highly expressed splice isoform transcript I (SIT1) and a short and weakly expressed splice isoform transcript II (SIT2) in mouse DRG. *DS-lncRNA* was expressed highly in DRG neuronal nuclei, but not in adjacent satellite cells. Peripheral nerve injury down-regulated the expression of *DS-lncRNA* and its two transcripts in injured DRG neurons. Rescuing this down-regulation attenuated the development and maintenance of neuropathic pain. Mimicking this down-regulation led to neuropathic pain-like behaviours in mice with the absence of peripheral nerve injury. Our findings suggest that DRG *DS-lncRNA* downregulation is required for neuropathic pain induction and maintenance.
This work was supported by NIH R01NS117484.

Disclosures: G. Govindarajalu: None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.03

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS113965
NIH Grant R01NS105715

Title: Sex specific role of RNA-binding protein, AUF1, on somatosensory responses from muscle after repetitive ischemia with reperfusion injury

Authors: *M. QUIJAS[1], A. A. WEYLER[1], L. F. QUEME COBAR[1,2], D. P. JOSHI[1], M. P. JANKOWSKI[1,2,3];
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Abstract: Musculoskeletal pain is experienced worldwide more than any other type of pain. Currently, few effective measures are available that help prevent acute pain from developing into

chronic pain. A major cause of disease-based myalgia is equated with repetitive ischemia with reperfusion (IR) injury. IR injury occurs in many disorders such as fibromyalgia or complex regional pain syndrome (CRPS) which are reported in significantly greater numbers in females than males. Pain tolerance is also lessened in females compared to males in these conditions. Mechanisms of sensitization that generate sex differences have been linked to distinctions in cytokine signaling. We recently identified an association between sensitization in group III and IV muscle afferents and an increase in acid sensing ion channel (ASIC) expression in male dorsal root ganglia (DRG) and an increase in TRPV1 expression in female DRGs that each correlated with behavioral hypersensitivity to IR. Both male and female DRGs displayed upregulation of the interleukin 1β (IL1β) receptor, IL1r1, in the DRGs after a single IR injury, but females specifically upregulated IL1r1 after double IR. To determine possible mechanisms by which distinct sex specific gene expression patterns modulated IR-related hypersensitivity in mice, we performed unbiased and targeted screening strategies in male and female DRGs with or without IR. Several distinct proteins were found to be differentially expressed between male and female DRGs including phosphorylated AU-rich element RNA binding protein 1 (AUF1), which was specifically upregulated in females DRGs when compared to males. AUF1 can stabilize or degrade RNA depending on cell type. Since AUF1 is known to have a high number (7) of IL1R1 binding sites in its 3'UTR, we hypothesized that AUF1 can promote the continued expression of IL1R1 and lead to prolonged hypersensitivity in females with repeated IR. To test this, we performed nerve targeted AUF1 knockdown in males and females with repeated IR injury and assessed behavioral responsiveness and gene expression with experimenters blinded to the conditions. No effect of AUF1 knockdown was observed on male DRG gene expression, or behavior post-repeated IR injury. AUF1 knockdown in females however, reduced the injury related paw guarding behaviors and prevented the upregulation of IL1r1 and TRPV1 in the DRGs. Data suggests that RNA binding proteins like AUF1 may underlie the sex specific effects on DRG gene expression that modulate behavioral hypersensitivity after repeated IR injury. This study may aid in finding distinct receptor differences related to the evolution of acute to chronic pain development between sexes.

Disclosures: M. Quijas: None. **A.A. Weyler:** None. **L.F. Queme Cobar:** None. **D.P. Joshi:** None. **M.P. Jankowski:** None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS105715
NIH Grant R01NS113965

NIH Fellowship 1F31NS122494-01

Title: Early life neuroimmune interactions modulate neonatal nociceptive priming.

Authors: *A. J. DOURSON[1], A. M. WARSHAK[1], M. C. HOFMANN[1], M. P. JANKOWSKI[1,2,3];
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Abstract: Peripheral injury evokes responses from a number of cellular systems including the nervous system and the immune system which alerts the body of damage and modulates repair of the affected area, respectively. Despite evidence that the pediatric population is unique compared to adults and requires specialized care following a painful insult, we do not have a full understanding of how the immune and nervous systems change during development to distinctly modulate injury responses in children. Clinical and rodent data indicate that early life aversive events alter the development of the somatosensory system, predisposing subjects to persistent pain following a repeated injury later in life. This “priming” effect is at least partially maintained by the central nervous and immune systems, but the peripheral component is unknown. We aimed to fill this gap in knowledge by investigating how peripheral immune cells may affect responses to repeated injury. Evidence suggests that macrophages are important mediators for acute pain, and can become “trained” by a previous stimulus. We hypothesized that developing macrophages uniquely drive neonatal nociceptive priming.
Neonatal hindpaw muscle incision induces pain-like behaviors and an inflamed microenvironment which includes damage-associated molecular patterns (DAMPs) that act on immune cells. Similar to previous work, we demonstrated that a postnatal day 7 (P7) incision “primes” the animals to an injury later in life indicated by exaggerated paw guarding and mechanical hypersensitivity compared to sham surgery controls. Macrophage depletion using macrophage fas-induced apoptosis (MaFIA) animals, blocked acute neonatal mechanical hypersensitivity and increased pro- and anti-inflammatory factors including nerve growth factor (NGF), interleukin 6 (IL-6) and IL-10. Further, the neonatal depletion of macrophages rescued the priming effect of early life injury. Using ATAC-seq, we found differences in chromatin accessibility in immature macrophages, an effect that may be modulated by early life incision and underlie how early life neuroimmune interactions modulate neonatal priming effects. Finally, adult bone marrow derived macrophages from neonatal incised animals were found to have enhanced production of pro-inflammatory cytokines upon stimulation with LPS/IFN-γ, indicating that the stem cell pool may be affected by early life injury. Together, these data suggest that early life injury alters how peripheral macrophages respond to repeated injury. Results may provide new information on how acute neonatal pain transitions to chronic pain after surgical injury.

Disclosures: A.J. Dourson: None. **A.M. Warshak:** None. **M.C. Hofmann:** None. **M.P. Jankowski:** None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.05

Topic: D.03. Somatosensation – Pain

Support: NCI R25-CA134283.

Title: Expression of neuronal injury markers after chemoradiation treatment in a rat model of radiation-induced swallowing dysfunction

Authors: *S. N. KING[1], N. KAISSIEH[1], A. DESHMUKH[2], E. GREENWELL[1], J. FOX[3], M. SHOJAEI[3], M. BLACKBURN[3], J. PETRUSKA[4];
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Abstract: Concurrent radiation and chemotherapy is the standard of care for the management of locally advanced oropharyngeal cancers. Impairments in swallowing function (dysphagia) is a dose-limiting toxicity of these radiation-based treatments that leads to negative effects in quality of life and oral intake. To develop targeted therapies to improve swallowing outcomes relies on understanding of the mechanisms that impact motor function after chemoradiation. The purpose of this study was to determine if chemoradiation damages axons/nerves that innervate the swallowing muscles, which could lead to impairments in sensorimotor control. We hypothesized that radiation given in combination with cisplatin chemotherapy will induce injury/stress-like markers i.e. activating transcription factor 3 (ATF-3) in swallow-related cranial nerves located within or outside the field of radiation. Twelve rats underwent 48Gy of fractionated radiation to the submental muscles using a clinical linear accelerator given in 12 fractions of 4Gy. Irradiated rats received either cisplatin 1mg/kg or saline IP once per week for four weeks during radiation treatment. One-month post treatment, the branch of the hypoglossal nerve, vagal ganglia, dorsal root ganglia, and trigeminal ganglia tissues were harvested. RNA was isolated and expression of injury markers were analyzed via RT-PCR. Alterations in ATF3 mRNA expression were observed. Results demonstrate that chemoradiation, which can induce functional deficits in swallowing, also provokes neuronal injury/stress markers.

Disclosures: S.N. King: None. **N. Kaissieh:** None. **A. Deshmukh:** None. **E. Greenwell:** None. **J. Fox:** None. **M. Shojaei:** None. **M. Blackburn:** None. **J. Petruska:** None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.06

Topic: D.03. Somatosensation – Pain

Support: CIHR - Vanier Fellowship to SMC

Title: Identifying the molecular mechanism for the pain caused by lionfish venom

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Abstract: The lionfish (Pterois volitans) is a venomous species endemic to the Indo-Pacific, but that has now invaded the Northwestern Atlantic as well as the Caribbean and Mediterranean Seas. It poses a growing health problem due to the increase in frequency of painful stings that it delivers, for which no treatment or antidote exists. A prior collaborative study by our group characterized the pain and inflammation caused by the lionfish venom and provided the first insight into the venom's cellular target in the peripheral nervous system - nonpeptidergic nociceptors. The aim of this research project is to isolate the algogenic toxin in the venom and identify its receptor target to understand the molecular mechanism for the pain caused by lionfish venom. The toxin will be isolated using High Pressure Liquid Chromatography (HPLC) to fractionate the venom, fractions will then be screened for their ability to elicit a rise in intracellular calcium in iPSC-derived sensory neurons using a high throughput screening system (FLIPR). Active fractions will be sub-fractionated and further screened until a single active fraction remains, this will then subject to mass spectrometry in order to identify the sequence of the algogenic toxin. Preliminary results point to the venom's receptor target, which has been verified using calcium imaging, behaviour and patch-clamp electrophysiology experiments. We have cloned the venom's target from Gymnothorax favagineus (Honeycomb moray eel), a predator of the lionfish, as well as from the lionfish itself to evaluate these targets' sensitivity to lionfish venom using calcium imaging and electrophysiology. Sequences of the human, G. favagineus and P. volitans genes have been aligned to identify divergent domains with an aim to generate chimera of this receptor to demonstrate whether insensitivity to lionfish venom can be conveyed to the human receptor by simply swapping domains with those from G. favagineus' or P. volitans' sequence. These results will 1) identify novel modulators of the pain pathway, 2) provide insights into the parallel evolution of the algogenic toxin in lionfish venom and its target in predators and 3) potentially produce novel analgesics by using low-dose toxin peptides in therapeutic agents to produce analgesic effects.

Disclosures: S. Mouchbahani-Constance: None. **R. Sharif-Naeini:** None.

Poster

P381. Pain: Nociceptors: Cell and Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P381.07

Topic: D.03. Somatosensation – Pain

Support: NS115209
GSU 2CI Neurogenomics

Title: Experience-dependent plasticity of cold nociception in Drosophila larvae

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Abstract: Organisms encounter constantly changing sensory stimuli requiring timescale-specific processing to ensure proper growth, development, and survival. The predictive qualities of many stimuli are encoded via inherent neuronal circuitry, but some are modified through exposure. While experience-dependent plasticity has been previously studied in a variety of modalities and sensory systems, little is known about thermal sensation, specifically cold. *Drosophila* larvae predominantly respond to acute noxious cold (≤10°C) with a full body contraction (CT) behavior requiring activation of Class III multidendritic neurons (CIIIs) by cold stimuli. Injury-induced cold sensitization via UV damage leads to behavioral plasticity such that CT responses are diminished, while body rolling behavior, which is normally associated with noxious heat, mechanical or chemical stimulation mediated by Class IV neurons (CIVs), begins to emerge. These data reveal that cold nociceptive circuitry exhibits injury-induced behavioral plasticity, however, whether this plasticity holds true in a non-injury model involving activity-dependent plasticity through accumulated cold experience remains unknown. Here, we sought to determine the existence and extent of cold-evoked behavioral plasticity as a result of prior cold experience under non-damage conditions and to explore the potential cellular mechanisms underlying changes in behavior. *Drosophila* larvae were reared under noxious cold temperature (10° C) for various durations prior to being selected at the 3rd instar stage for behavioral testing during acute cold challenge at noxious and innocuous temperatures. In a set of parallel experiments, chronic "fictive cold" was achieved by targeted optogenetic stimulation of CIII neurons using the GAL4/UAS system to selectively express the light-gated cation channel, ChETA. In this condition, larvae were reared *sans* cold at room temperature (23°C) while being exposed to repeated blue light stimulation in a custom built, high-throughput device. We find that prior cold experience causes cold-evoked behavioral plasticity, exhibited by increased magnitude of CT behavior as well as the appearance of additional CIV-related behaviors such as C-bending, a precursor to rolling. These changes in behavioral responses were mirrored in "fictive" cold reared larvae, indicating that chronic activation of primary nociceptors is sufficient to induce behavioral sensitization and shifts. Together, these data demonstrate that the cold nociceptive system is capable of experience-dependent plasticity in non-injury conditions and that this change is neuronally driven.

Disclosures: K.J. Donaldson: None. **J.T. Papania:** None. **D.N. Cox:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.01

Topic: D.03. Somatosensation – Pain

Support: NINDS: R01 NS099245

Title: Activity in the parabrachial nuclear complex during neuropathic pain in awake, behaving mice

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Abstract: Activity in the parabrachial nuclear complex during neuropathic pain in awake, behaving mice
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The parabrachial nuclear complex (PB) is a key nexus for aversive behavior, including that related to pain perception. We have previously shown that, during chronic pain, PB activity is amplified, and its neurons respond to noxious stimuli with prolonged after-discharges, defined as responses that far outlast the stimulus. The activity of PB neurons in pain conditions has been studied almost exclusively in anesthetized animals. Because pain and aversion have important affective components, and because they are readily modulated by behavioral states, we seek to study PB in awake animals. We developed a method to investigate PB in awake, behaving mice by obtaining single unit *in vivo* electrophysiological recordings from head restrained mice. This allows us to record for extended periods of time, up to 2 hours, responses to different stimuli, under different conditions. This approach also allows us to compare, in the same animals, PB activity before the induction of chronic pain, while chronic pain develops, and after it has been established. Here, we compare single-unit PB data from naïve mice, both males and females, with activity during chronic pain.

Disclosures: J. Smith: None. **Y. Ji:** None. **R. Masri:** None. **A. Keller:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.02

Topic: D.03. Somatosensation – Pain

Support: NIH T32 GM008600
NIH R21 NS121946

Title: Sting activation in microglia attenuates nerve injury-induced neuropathic pain

Authors: A. S. ANDRIESSEN[1], A. S. PRUDENTE[2], J. ZHAO[1], A. MCGINNIS[1], R.-R. JI[1], A. G. NACKLEY[1], T. BERTA[2], ***C. R. DONNELLY**[1];
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Abstract: Interactions between central glial cells and neurons are critical to the pathogenesis of neuropathic pain. Peripheral nerve injuries, such as chronic constriction injury (CCI) of the sciatic nerve, induce profound changes in the morphological, molecular, and functional properties of spinal microglia, which act to promote sensitization of neurons in pain circuitry, thereby driving the initiation and maintenance of chronic pain. We recently demonstrated that activation of stimulator of interferon genes (STING) in peripheral sensory neurons could attenuate pain in naïve mice and in a variety of murine models, including CCI-induced neuropathic pain. However, the prolonged antinociceptive effects of STING agonists were only partially mediated by STING signaling in sensory neurons, suggesting an important role of STING signaling in additional cell types. In naïve mice, we found that the majority of cells expressing STING in the spinal dorsal horn (SDH) were Iba1+ cells (~90%), likely microglia. Based on these data, we sought to determine the contribution of STING signaling in microglia to CCI-induced neuropathic pain using *Sting1*$^{fx/fx}$; *Cx3cr1*-Cre/ER^{T2} conditional knockout (STING-cKO^{MG}) mice. Notably, we observed that repeated intrathecal (e.g. spinal) administration of small molecule STING agonists immediately after CCI (days 3-5) produced a robust, but transient reduction in neuropathic pain behaviors, whereas administration at later timepoints (days 11-13) led to sustained antinociception and early resolution of pain. Although the acute antinociceptive effects of STING agonists were maintained in STING-cKO^{MG} mice, the long-term analgesic effects were abolished. Moreover, genetic and pharmacologic ablation of microglia could also abolish the prolonged antinociceptive effects of STING agonists in naïve and CCI mice. Finally, adoptive transfer of microglia from STING-WT, but not STING-cKO^{MG}, mice following incubation with STING agonists produced long-lasting antinociceptive effects in multiple models of neuropathic pain. Ongoing work is aimed at understanding the mechanisms by which STING activation in microglia attenuates pain by altering the neuroinflammatory microenvironment induced by peripheral nerve injury.

Disclosures: A.S. Andriessen: None. **A.S. Prudente:** None. **J. Zhao:** None. **A. McGinnis:** None. **R. Ji:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); R.R.J. submitted a patent related to this study:. F. Consulting Fees (e.g., advisory boards); R.R.J. is a member of Boston Scientific advisory board. These activities are unrelated to this study.. **A.G. Nackley:** None. **T. Berta:** None. **C.R. Donnelly:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.R.D. submitted a patent related to this study:.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.03

Topic: D.03. Somatosensation – Pain

Title: Hyperexcitation of prodynorphin neurons in the prelimbic cortex in a mouse model of surgical pain

Authors: *S. ZHOU, P. L. SHEETS;
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Abstract: Incomplete understanding of how surgical incision elicits maladaptive changes to endogenous opioid circuits in the central nervous system remains a significant hurdle in developing new therapeutics for alleviating surgical pain. **Objective**: Determine how surgical incision alters endogenous opioid circuits in the medial prefrontal cortex (mPFC). **Rationale:** Prodynorphin (Pdyn) is the precursor peptide for the endogenous opioid dynorphin and is expressed in brain areas implicated in pain regulation. Increased expression of Pdyn mRNA is observed in the mPFC in a mouse model of chronic pain. One important subdivision of mPFC is the prelimbic cortex (PL). While maladaptive changes to the PL have been reported in multiple chronic pain models, intrinsic and synaptic alterations to PL Pdyn neurons following surgical incision are not known. **Methods:** Female Pdyn-IRES-Cre (B6;129S-$^{Pdyntm1.1(cre)Mjkr}$/LowIJ; stock #027958; Jackson labs) mice were mated with male Ai14 (B6;129S6-Gt(ROSA)$^{26SORtm14(CAG-tdTomato)Hze}$/J; stock #007914; Jackson Labs) mice to label Pdyn neurons with tdTomato. Coronal brain slices from offspring showed that Pdyn neurons are predominantly expressed in PL laminae 2/3 (L2/3). Here, we performed whole-cell patch clamp recordings in acute brain slices to study changes to L2/3 Pdyn neurons elicited by the plantar incision model of surgical pain (PIM). **Results:** Both male (n = 10) and female (n = 10) mice displayed mechanical allodynia compared to sham controls (male, n=11; female, n=9) one day after plantar incision. Electrophysiology data show that L2/3 PL Pdyn neurons are an intrinsically heterogenous population consisting of pyramidal and inhibitory-like neurons, which we confirmed morphologically. Interestingly, only pyramidal L2/3 PL Pdyn neurons display hyperexcitability

one day after plantar incision ($p < 0.05$). There is also a decrease in the frequency of spontaneous inhibitory post-synaptic currents in pyramidal Pdyn neurons in male PIM mice. We are currently exploring the role of PL Pdyn neurons in regulating surgical pain behaviors by using *in vivo* optogenetics. By manipulating the activity of PL Pdyn neurons, we will study the role of PL Pdyn neurons in regulating surgical pain behaviors. **Conclusion:** PL Pdyn neurons are a heterogenous population, and the excitability of only pyramidal Pdyn neurons is increased after plantar incision in both male and female. Data from male mice suggest that increased excitability is a result of reduced inhibitory synaptic inputs. We are currently studying the functional role of Pdyn neurons in surgical pain regulation, and the origin of the decrease in inhibitory inputs to Pdyn neurons in PIM mice.

Disclosures: S. Zhou: None. **P.L. Sheets:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant T-32 NS076401

Title: Behavioral and functional neural correlates of visual hypersensitivity in chronic nociplastic pain

Authors: *N. C. WALLER, I. MAWLA, E. ICHESCO, A. SCHREPF, S. E. HARTE, R. E. HARRIS;
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Abstract: Fibromyalgia (FM) is a nociplastic pain condition with amplified central nervous system responses to external stimuli resulting in symptoms of sensory hypersensitivity and hyperalgesia. This hypersensitivity extends to non-painful stimuli including lights, which suggests multimodal central sensory amplification. The present study sought to understand behavioral and neural responses to aversive visual stimulation in individuals with FM compared to healthy controls (HC) using quantitative sensory testing (QST) and neuroimaging. Female participants (Total N = 26; FM: N=13) underwent aversive visual stimuli presentations of black/white checkerboard patterns. Visual stimuli were presented pseudo-randomly in 6 brightness levels. Participants rated each stimulus on Gracely-Box Scales for sensory intensity and unpleasantness. Sensory sensitivity to visual stimuli were analyzed with repeated measures ANOVA. Functional images were preprocessed in fMRIPrep and analyzed via FSL Local Analysis of Mixed Effects contrasting visual and rest conditions ($Z = 2.3$, $p < 0.05$). Whole-brain linear regression analyses assessed the associations between the images and various clinical

measures. Individuals with FM and HCs reported greater perceived sensory unpleasantness and intensity in a step-wise manner with increasing brightness intensity levels (HC: unpleasantness, $F(5) = 9.945$, $p < 0.001$; intensity, $F(5) = 9.465$, $p < 0.001$; FM: unpleasantness, $F(5) = 20.152$, $p < 0.001$; intensity, $F(5) = 16.357$, $p < 0.001$). Compared to HCs, individuals with FM rated visual stimuli as more unpleasant and intense compared to HCs (unpleasantness, $F(1, 21) = 6.690$, $p = 0.017$; intensity, $F(1, 21) = 5.212$, $p = 0.033$). fMRI contrast analyses between visual stimulation and rest revealed increased activity in visual and pain processing regions in individuals with FM to HCs, including bilateral dlPFC and V1. This difference was driven primarily by increased regional activation during rest. Whole-brain regression analyses in individuals with FM revealed positive correlations between visual-rest comparisons of pain severity and IFG, among others. (all $r > 0.69$, $p = 0.05$). Consistent with previous findings, individuals with FM reported significantly increased unpleasantness and intensity ratings to aversive visual stimulation compared to HCs. FM participants also showed increased brain activation during visual stimulation in core visual and pain network regions, including the dlPFC which is implicated in antinociceptive processing. These findings suggest increased antinociceptive network reactivity in patients with nociplastic pain occurs in nonpainful sensory contexts.

Disclosures: N.C. Waller: None. **I. Mawla:** None. **E. Ichesco:** None. **A. Schrepf:** None. **S.E. Harte:** None. **R.E. Harris:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.05

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R35GM137906
Rita Allen Foundation
Foundation for Anesthesia Education and Research (FAER) Mentored Research Training Grant

Title: Transcriptome analysis of repopulated spinal cord microglia identifies potential therapeutic targets in a mouse model of complex regional pain syndrome

Authors: *L. J. DONOVAN, C. M. BRIDGES, N. A. HUCK, S. WU, T. E. FORMAN, C. E. JORDAN, A. S. GARDNER, E. S. HAIGHT, V. L. TAWFIK;
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Abstract: Complex regional pain syndrome (CRPS) is a form of chronic pain, occurring after limb fracture and immobilization, that affects 50,000 new patients per year in the United States alone. CRPS exhibits a clear acute-to-chronic transition and as a result rodent models of CRPS

can be utilized to understand mechanisms underlying the development of chronic pain. In particular, we have found prolonged microglial activation in the spinal cord dorsal horn in a rodent tibial fracture/casting model of CRPS which peaks at the time of acute-to-chronic pain transition. A more detailed understanding of the contribution of microglial activation to CRPS progression would enable the development of successful glial-targeted therapeutics. We hypothesized that diphtheria toxin-mediated transient depletion of microglia (and not macrophages) at 3 weeks post-fracture in *Cx3CR1*^{CreER}; *R26*^{iDTR} mice would resolve existing pain in males but not females after tibial fracture/casting. We further hypothesized that the transcriptome of newly repopulated microglia would reveal key genes that contribute to the resolution of pain. We performed unilateral tibial fracture followed by 3 weeks of casting in male and female *Cx3CR1*^{CreER}; *R26*^{iDTR} mice. To deplete microglia, diphtheria toxin was injected at the time of cast removal, at the time of acute-to-chronic transition. Mouse behavioral tests were performed after cast removal. Microglial cells from the spinal cord were isolated 2 weeks after depletion/repopulation (5 weeks post-fracture) by fluorescence-activated cell sorting (FACS) and the transcriptome was profiled using RNA sequencing (RNA-seq). Microglial depletion reversed existing mechanical allodynia and thermal hyperalgesia in both males and females. RNA-seq performed on FACS-isolated lumbar spinal cord microglia showed that the microglial transcriptome profiles clustered by group, with microglia depletion/repopulation having the greatest effect. DESeq2 analysis uncovered a shared set of differentially expressed genes (DEGs) in males and females after microglial depletion. Further, weighted gene correlation network analysis (WGCNA) of the DEGs was used to identify modules most significantly associated with a pain state vs no-pain state using module-trait correlations. Overall, transcriptome analysis of repopulated microglia identified a number of microglial genes and pathways that may contribute to the transition from acute-to-chronic pain in a CRPS model. Future studies will focus on analyzing lead genes as potential therapeutic targets.

Disclosures: L.J. Donovan: None. **C.M. Bridges:** None. **N.A. Huck:** None. **S. Wu:** None. **T.E. Forman:** None. **C.E. Jordan:** None. **A.S. Gardner:** None. **E.S. Haight:** None. **V.L. Tawfik:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.06

Topic: D.03. Somatosensation – Pain

Support: NIH grant NS101880
NIH grant GM120844

Title: Theta-burst stimulation induces spinal cord long-term potentiation and pain hypersensitivity via α2δ-1-bound NMDARs

Authors: *Y. HUANG, S.-R. CHEN, H. CHEN, J.-J. ZHOU, D. JIN, H.-L. PAN;
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Abstract: Activity-dependent long-term potentiation (LTP) is a fundamental feature of synaptic plasticity, and spinal cord LTP represents a state of central sensitization in chronic pain. Tetanic high-frequency stimulation (HFS) has been used to induce LTP in the spinal cord. However, many painful conditions generate theta rhythm bursting activity in primary afferent nerves. In this study, we determined how theta-burst stimulation (TBS) impacts LTP in the spinal dorsal horn and pain hypersensitivity. TBS induced LTP in 50% of excitatory neurons expressing vesicular glutamate transporter 2 (VGluT2) but only in 16% of inhibitory neurons expressing vesicular GABA transporter (VGAT). Nerve injury significantly increased the ratio of VGluT2-expressing, but not VGAT-expressing, neurons that showed TBS-induced LTP. TBS-induced LTP in VGluT2-expressing neurons was diminished by the NMDAR antagonist AP5, and was partially reduced in mice with conditional knockout of GluN1 in primary sensory neurons. Also, TBS failed to induce LTP in VGluT2-expressing neurons in the presence of α2δ-1 inhibition with gabapentin, genetic α2δ-1 knockout, or disruption of the α2δ-1-NMDAR interaction with α2δ-1Tat peptide. Furthermore, TBS of the sciatic nerve induced long-lasting allodynia and hyperalgesia in wild-type, but not in α2δ-1 knockout mice. In addition, treatment with the NMDAR antagonist memantine or AP5, gabapentin, or α2δ-1Tat peptide diminished TBS-induced allodynia and hyperalgesia. These findings indicate that TBS induces pain hypersensitivity and LTP predominantly in spinal excitatory dorsal horn neurons via activation of α2δ-1-bound NMDARs.

Disclosures: Y. Huang: None. **S. Chen:** None. **H. Chen:** None. **J. Zhou:** None. **D. Jin:** None. **H. Pan:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS104705

Title: Glun2d subunit-containing nmda receptors mediate cisplatin-induced neuropathic pain

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Abstract: Augmented N-methyl-D-aspartate receptor (NMDAR)-dependent synaptic plasticity at spinal as well as cortical levels is believed to contribute to enhanced sensory responses after chronic pain, but subunit specific NMDAR involvement in chronic pain is still unclear. Interestingly GluN2D subunit-containing NMDARs could be a potential target for chronic pain, because of their unique biophysical and pharmacological properties (less sensitivity to Mg^{2+}-block, prolong deactivation kinetics and higher glutamate/glycine affinity). Therefore, in the present study we used the global GluN2D knockout mouse model to characterize its potential role in inflammation and cisplatin induced neuropathic pain. We used three different mouse models to assess the pain (acute pain induced by 5% formalin, chronic pain induced by Complete Freund's adjuvant (CFA), neuropathic pain induced by the cisplatin), We found that formalin and CFA injected wildtype and global GluN2D KO mice showed similar pain sensitivity. In contrast, cisplatin injected global GluN2D KO mice had attenuated mechanical hypersensitivity compared to wildtype mice. Additional results using pharmacological tools further supported these findings. Overall, these results suggest that GluN2D subunit-containing NMDARs are involved in neuropathic pain.

Disclosures: D. Gawande: None. **R. Pavuluri:** None. **S.G. Gakare:** None. **L. Jenson:** None. **S. Dravid:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant GM115384

Title: Ampakines relieves persistent neuropathic pain through the restoration of anti-nociceptive response in the prefrontal cortex in rats

Authors: *Y. LIU, R. TALAY, M. MICHAEL, I. FRIESNER, F. ZENG, H. XU, G. SUN, Z. CHEN, Q. ZHANG, J. WANG;
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Abstract: While the prelimbic prefrontal cortex (PL-PFC) has been shown to play a key role in the top-down regulation of sensory pain, few studies have examined pharmacological agents that target maladaptive changes in the region for chronic pain regulation. AMPAkines, compounds that enhance glutamate transmission by binding to the AMPA (Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor, have been shown to relieve acute incisional and inflammatory pain symptoms in animals. However, the cortical mechanisms for their anti-nociceptive effects remain unknown. Here, we studied the impact of AMPAkines on the anti-nociceptive function of

neurons in the PL-PFC in a well-known neuropathic pain model. Silicon probes were implanted in the PL-PFC for *in vivo* recording of neuronal spike activities in response to peripheral noxious (pin prick) or non-noxious (von Frey (VF) filaments) stimuli from freely moving Sprague-Dawley rats. The impact of chronic neuropathic pain induced by spared nerve injury (SNI) was evaluated by basal and peak firing rates extracted from each individual neuron's perievent time histograms. We then studied how the PL-PFC neuronal abnormality in the chronic pain state was rescued by systemic delivery of a well-established AMPAkine (CX546). The chronic pain relief effects from systemic and intracranial administration of CX546 were compared using both mechanical and cold allodynia tests. We used optogenetic and pharmacological modulations of the PL-PFC neurons to verify whether CX546 could target PL-PFC AMPA receptors to relieve pain. In both allodynia tests, we interfered PL-PFC through either shining yellow light on halorhodopsin-expressing pyramidal neurons or injecting AMPA receptor antagonist (NBQX) intracranially. We found that neurons in the PL-PFC increase their firing rates in response to noxious stimulations. While chronic pain suppresses this pain response, CX546 restores the anti-nociceptive response in PL-PFC neurons to reduce chronic pain. Furthermore, we found that both systemic and direct delivery of CX546 into the PL-PFC inhibited chronic pain symptoms, whereas optogenetic and pharmacological inhibitions of PL-PFC neurons blocked the anti-nociceptive effects of CX546. The analgesic and respiratory-stimulating properties of AMPAkines make them an intriguing treatment choice for persistent neuropathic pain. More generally, our results indicate that drugs targeting the endogenous cortical pain-regulatory system are likely to have important roles in the treatment of chronic pain.

Disclosures: Y. Liu: None. **R. Talay:** None. **M. Michael:** None. **I. Friesner:** None. **F. Zeng:** None. **H. Xu:** None. **G. Sun:** None. **Z. Chen:** None. **Q. Zhang:** None. **J. Wang:** None.

Poster

P401. Mechanisms of Neurophatic Pain II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P401.09

Topic: D.03. Somatosensation – Pain

Support: U.S. Army Medical Research and Materiel Command Grant W81XWH-16-1-0717
Rita Allen Foundation Award in Pain
University of Texas System Rising STARs Award
University of Texas MD Anderson Cancer Center start-up funds
American Australian Association Sir Keith Murdoch Fellowship

Title: Antibody receptor signaling from spinal cord astrocytes promotes neuropathic pain

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Abstract: Background: Injury to somatosensory nerves can lead to mechanical allodynia, in which normally innocuous touch is perceived as painful. The cellular and molecular mechanisms responsible for mechanical allodynia are not fully understood, and therefore therapeutic options remain inadequate. Inflammatory signaling arising from spinal cord glial cells are thought to promote pain hypersensitivity, but it remains unclear what mechanisms control spinal glia activation after injury. Previous transcriptional datasets identified Fc gamma receptors (FcγR), the antibody receptors for immunoglobulin G (IgG), as a potential signaling axis associated with mechanical allodynia. Here, we demonstrate a role for Fc gamma receptor subtype IIa (FcγRIIa) on spinal cord astrocytes as contributing to neuropathic pain.
Methods: To model neuropathic pain, adult male and female Sprague Dawley rats received unilateral chronic constriction injury (CCI) of the sciatic nerve. To determine the role of spinal FcγRs signaling on pain behaviors, we used intrathecal (i.t.) injections of IgG immune complex (IgG-IC). To determine the functional role of FcγRIIa$^+$ astrocytes *in vivo*, we used a CRISPR-Cas9 approach to edit *Fcgr2a* function by i.t. injection of viral constructs co-expressing GFAP-driven Cas9 (or eGFP as control) and single guide RNA (sgRNA) against *Fcgr2a*. Pain-related behaviors were assessed by measuring reflexive withdrawal responses to innocuous punctate stimuli (von Frey filaments) and dynamic light touch (paintbrush assay). Gene and protein expression were measured by *in situ* hybridization, qRT-PCR, and immunohistochemistry. For *in vitro* experiments, astrocytes were purified by immunopanning and treated with IgG-IC.
Results: Activating FcγRs by spinal administration of IgG-IC is pro-nociceptive. *Fcgr2a* expression increases in ipsilateral spinal dorsal horn after CCI, and FcγRIIa co-localizes primarily with GFAP$^+$ astrocytes. Astrocytes respond to IgG-IC by increasing production of pro-inflammatory mediators, including TNF and IL-1β, which are known to influence nociceptor activity. Genetic disruption of *Fcgr2a* in spinal GFAP$^+$ astrocytes attenuates mechanical allodynia after CCI.
Conclusion: These data suggest FcγRIIa on spinal astrocytes may be activated following peripheral nerve injury and directly contribute to injury-induced tactile pain. These findings expand our understanding of how glial neuroimmune signaling contributes to mechanical allodynia and suggest that autoantibody IgG signaling at glial FcγRs could be a novel therapeutic target to alleviate suffering from neuropathic pain.

Disclosures: M.J. Lacagnina: None. **K. Willcox:** None. **J. Li:** None. **A.G. Alfaro:** None. **S. Lorca:** None. **P.M. Grace:** None.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.01

Topic: D.06. Auditory & Vestibular Systems

Title: Examining the role of inflammation in a zebrafish model of sensory hair cell aging

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Abstract: Thirty-six million Americans suffer from hearing impairment, resulting in significant decreases in quality of life for those affected. Over 50% of individuals above the age of 75 experience age-related hearing loss (ARHL), and as the Baby Boomer cohort ages an increasing portion of the population will require assistive listening devices. While assistive technologies improve hearing capabilities in some, it is of great interest to prevent ARHL and preserve hearing. We proposed that two likely primary causes of ARHL are localized inflammation and mitochondrial dysfunction; here we focus on the role of inflammation in a zebrafish model. Zebrafish are an excellent model for auditory research, particularly studies of sensory hair cell development and physiology, due in part to the presence of exterior lateral line hair cells which are homologous to the hair cells in the human inner ear. Additionally, zebrafish development occurs rapidly and lateral line hair cells are in a constant state of turnover, resulting in lateral line organs containing cells of different ages. Lateral line hair cells are relatively mature in five days-post fertilization (dpf) animals, allowing us to study hair cell aging in a period of days, rather than waiting years for an animal to age. We hypothesize that inhibiting inflammation will prolong the functional lifespan of lateral line hair cells, and thus we would expect reduced hair cell addition and decreased longevity under inflammatory conditions. Using transgenic zebrafish that express green fluorescent protein (GFP) in hair cells, we pharmacologically manipulated a key inflammatory cytokine TNF-alpha and global inflammation using dexamethasone and lipopolysaccharide to investigate hair cell longevity *in vivo*. Fish were incubated in 4',6-diamidino-2-phenylindole (DAPI) at 4 dpf to label existing hair cells, then incubated in one of our inflammatory modulators. We can discriminate between cells that were present during at 4 dpf and newly added cells, as older cells are GFP+ and DAPI+, while newer cells are only GFP+. Our preliminary data demonstrates that inhibiting TNF-alpha increases hair cell longevity, consistent with our hypothesis. Future work will include genetic manipulation of inflammatory signaling and examine the role of mitochondria in the hair cell aging process. This work will increase understanding of inflammatory signaling in ARHL and may yield molecular targets for therapeutic development.

Disclosures: E. Dale: None. **O. Molano:** None. **A.B. Coffin:** None.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.02

Topic: D.06. Auditory & Vestibular Systems

Support: WSU Vancouver Mini-grant

Title: Effects of high fat diet on aminoglycoside-induced hearing damage, regeneration, and gut microbiome composition in zebrafish

Authors: *S. SCHLOSS[1], I. HINCHLIFF[2,4], K. J. LAWTON[3], C. A. GAULKE[5], A. B. COFFIN[6];
[1]Washington State University, Vancouver, Vancouver, WA; [3]Neurosci., [2]Washington State Univ. Vancouver, Vancouver, WA; [4]Camas High Sch., Camas, WA; [5]Pathobiology, The Univ. of Illinois Urbana-Champaign, Champaign, IL; [6]Washington State Univ., Vancouver, WA

Abstract: Obesity is an extremely prevalent health issue that affects almost half of all people in the United States, according to the CDC. The consumption of a high fat diet (HFD) and large amounts of processed foods not only results in weight gain but can lead to a host of other comorbid conditions, including diabetes and heart disease. One relatively unrecognized comorbid condition that can present alongside obesity is hearing loss. Hearing loss is often caused by damage to sensory hair cells in the inner ear but it's not known how obesity influences hair cell survival. Here we examine the correlation between diet-induced obesity and hair cell damage in a zebrafish model. Zebrafish have hair cells located on a sensory system called the lateral line and are a well-established model for hearing research, in part because fish can robustly regenerate hair cells. Similarly, zebrafish are an excellent model for diet-induced obesity. We fed zebrafish for one month using either a control diet of dry food, dry food mixed with 20% lard, or dry food mixed with 20% egg yolk powder. We used an aminoglycoside antibiotic to damage hair cells and compared counts between groups. We also examined hair cell regeneration 48 hours after aminoglycoside treatment. Obesity has been linked with an overproduction of reactive oxygen species (ROS) which may contribute to hair cell death via apoptosis. ROS could also damage hair cell precursors and result in decreased hair cell regeneration for the HFD groups. A link exists between diet and gut microbiome composition, so we also assessed whether differences in gut microbiome composition can be related to hearing loss. We tested this hypothesis by 16S sequencing fecal samples and quantifying microbial populations based on taxon-specific DNA signatures. The mechanism behind the relationship of diet-induced obesity and hearing loss is not understood. Our work establishes a model for future mechanistic studies.

Disclosures: S. Schloss: None. **I. Hinchliff:** None. **K.J. Lawton:** A. Employment/Salary (full or part-time):; Rewire Neuroscience. **C.A. Gaulke:** None. **A.B. Coffin:** None.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.03

Topic: D.06. Auditory & Vestibular Systems

Title: Identification and characterization of OTO-510 as a novel approach to the prevention of cisplatin-induced hearing loss

Authors: *P. D. MATHUR, C. FERNANDEZ, P. URIBE, R. FERNANDEZ, S. SZOBOTA, N. TSIVKOVSKAIA, X. WANG, A. JONES, S. SIEGEL, G. PETTUS, K. HALDERMAN, R. FONG, J. HOU, A. STEPHANEKO, E. ALLSOPP, B. JACQUES, F. PIU, A. FOSTER; Otonomy Inc., San Diego, CA

Abstract: Among the effective chemotherapeutics used in oncology, cisplatin remains the first line of treatment for various cancer types, despite its deleterious effects on the peripheral auditory system. Several human and animal studies have demonstrated that cisplatin causes damage to the sensory hair cells (HCs), spiral ganglion neurons (SGNs) and cells of the stria vascularis (SV) in the inner ear, leading to permanent and bilateral sensorineural hearing loss. Local administration of drugs to the inner ear is an attractive means for preventing cisplatin-induced ototoxicity and ensuring low systemic exposure that does not compromise the anti-tumor efficacy of intravenously administered cisplatin. Here we report local administration of OTO-510 as an effective otoprotection strategy against cisplatin-induced hearing loss (CIHL). Approximately 100 molecules from different potentially otoprotective classes were screened at multiple concentrations in cochlear explant assays utilizing tissues from neonatal rat pups to evaluate otoprotection in HCs, SGNs and SV cells against cisplatin-induced damage. This led to the identification of a novel therapeutic class of molecules with consistently greater effects in preventing cisplatin-induced damage to HCs, SGNs and SV cells than other otoprotective molecules reported in the literature and in clinical studies, such as STS. Selected molecules from this novel class were tested for their cisplatin binding ability at equimolar concentrations in vitro using HPLC. Top performing candidates were then formulated to optimize local delivery and their pharmacokinetic properties were evaluated. Intratympanic delivery of the lead molecule OMY-150 in an optimized formulation (OTO-510) provided therapeutic levels of OMY-150 to the inner ear compartment with minimal systemic exposure. To evaluate its *in vivo* efficacy, OTO-510 was delivered locally via intratympanic administration to rats prior to cisplatin dosing, and the degree of otoprotection was assessed by recording auditory brainstem responses (ABRs) across several frequencies and by histological assessment of inner ear cells. OTO-510 prevented deleterious hearing threshold shifts and preserved all inner ear structures *in vivo*. Thus, OTO-510 represents a novel approach for protection against cisplatin-induced damage by optimizing an effective otoprotectant for local delivery to the inner. These results support the continued development of OTO-510 for the prevention of hearing loss caused by cisplatin administration in cancer patients.

Disclosures: P.D. Mathur: A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **C. Fernandez:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **P. Uribe:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **R. Fernandez:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **S. Szobota:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **N. Tsivkovskaia:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **X. Wang:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **A. Jones:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **S. Siegel:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **G. Pettus:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **K. Halderman:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **R. Fong:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **J. Hou:** A. Employment/Salary (full or part-time):; Otonomy Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **A. Stephaneko:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **E. Allsopp:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **B. Jacques:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **F. Piu:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc. **A. Foster:** A. Employment/Salary (full or part-time):; Otonomy Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy Inc..

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.04

Topic: D.06. Auditory & Vestibular Systems

Title: Novel Fab fragments derived from trk-selective agonist monoclonal antibodies are potent and selective antagonists of the trkb and trkc receptors

Authors: *S. SIEGEL[1], P. MATHUR[1], S. SZOBOTA[2], A. C. FOSTER[3];
[1]Otonomy, San Diego, CA; [3]Res., [2]Otonomy, Inc., San Diego, CA

Abstract: The neurotrophin receptors, TrkA, TrkB, and TrkC are expressed in neurons throughout the nervous system and mediate trophic signaling such as neuron survival, neurite outgrowth, and synaptogenesis. We previously demonstrated that agonistic monoclonal antibodies (mAbs) raised against TrkB or TrkC produced potent neurotrophic effects in cultured cochlear neurons (Szobota et al., PLoSOne 2019). Here, we examined the function of the Fab fragments of these mAbs. Surprisingly, we found that the Fab fragments blocked neurotrophic signaling at their respective receptors, functioning as highly-selective and potent antagonists. In a cell line overexpressing human TrkB, the TrkB-selective Fab had no agonist activity by itself, but at nanomolar concentrations inhibited phosphorylation of TrkB by BDNF and blocked its downstream effects on ERK phosphorylation. Similarly, in a cell line overexpressing human TrkC, the TrkC-selective Fab had no agonist activity by itself, but at nanomolar concentrations inhibited phosphorylation of TrkC by NT-3 and blocked downstream phosphorylation of ERK. The Fab fragments were highly selective for their respective Trk receptor and did not activate or inhibit other Trk subtypes. To date, inhibitors of neurotrophin receptors have demonstrated poor selectivity for Trk subtypes. For example, anti-cancer agents that target the intracellular kinase domains of Trk receptors have demonstrated potent inhibition but little selectivity between subtypes due to the conserved nature of the kinase domains. Subtype-selective Fabs, which bind to targeted extracellular epitopes, provide a unique opportunity to dissect the physiological roles of TrkB and TrkC in cellular systems that present both of these receptors, such as cochlear neurons. Previously, we found that survival of dissociated rat cochlear neurons was greatly augmented by treatment with BDNF, NT-3, or mAb Trk agonists. Application of exogenous BDNF or a TrkB-selective mAb greatly enhanced survival. In order to directly assess the significance of both endogenous and exogenous BDNF signaling in cochlear neurons, we treated these cultures with the TrkB-selective Fab, which produced a dose-dependent inhibition of neuron survival both in the absence and presence of exogenous BDNF, indicating that TrkB signaling is critical in this model of cochlear neuron survival. The Fabs described here represent novel tools for understanding the role of TrkB and TrkC in the development, maintenance, and

restoration of neuronal systems in physiology and disease. Additional studies with these Fab antagonists will examine the specific roles of TrkB and TrkC in cochlear function and repair.

Disclosures: S. Siegel: A. Employment/Salary (full or part-time):; Otonomy. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy. **P. Mathur:** A. Employment/Salary (full or part-time):; Otonomy. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy. **S. Szobota:** A. Employment/Salary (full or part-time):; Otonomy. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy. **A.C. Foster:** A. Employment/Salary (full or part-time):; Otonomy. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.05

Topic: D.06. Auditory & Vestibular Systems

Title: Local delivery of BDNF (OTO-413) and a selective TrkB agonist (M3) restores hearing function in models of cochlear synaptopathy

Authors: C. FERNANDEZ, N. TSIVKOVSKAIA, X. WANG, R. FERNANDEZ, T. ALTMANN, P. URIBE, P. D. MATHUR, Y. TSABUR, S. SIEGEL, *S. SZOBOTA, A. STEPANENKO, B. JACQUES, A. C. FOSTER, F. PIU;
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Abstract: Evidence from preclinical and clinical studies indicates that loss of the synapses that connect inner hair cells of the cochlea with spiral ganglion neurons (SGNs), whose axons form the cochlear nerve, contributes to hearing impairment. This synaptopathy is proposed as an underlying pathology in age-related and noise-induced hearing deficits and has been hypothesized to explain speech-in-noise hearing difficulties that can occur despite normal audiometric thresholds. The application of Trk receptor agonists, in the form of neurotrophins such as Brain Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), or high affinity monoclonal antibodies (mAbs), has been investigated as a therapeutic approach for hearing loss based on their ability to provide trophic support to SGNs in the cochlea. In recent studies, BDNF, NT-3 and several Trk agonist mAbs were found to promote survival, neurite extension and synapse restoration in ex vivo cochlear cultures, with the strongest effects observed using the TrkB agonists BDNF and a TrkB-selective mAb, M3.
We developed sustained-exposure formulations of both BDNF (known as OTO-413) and M3 for

intratympanic delivery to the inner ear. Pharmacokinetic studies in rats demonstrated significant and dose-dependent inner ear drug levels for several weeks. Following noise exposure, administration of either BDNF or M3 one day post-trauma, improved functional (ABR, wave 1 amplitude) and histological (ribbon synapse counts) measures of hearing and cochlear synaptopathy. We further characterized the effects of OTO-413 when delivered up to 7 days post-noise insult and found that OTO-413 administration again improved these functional and histological readouts. In addition, biomarker studies revealed that elevated levels of proteins important for neuronal survival and synaptogenesis are present for several days in cochlear tissues following a single injection of OTO-413.
The present studies using sustained-exposure formulations of BDNF and M3 demonstrate the effectiveness of TrkB agonists in alleviating noise-induced cochlear synaptopathy in vivo. The pharmacokinetic and pharmacodynamic profiles, along with the practical benefits of delayed administration, create an attractive therapeutic approach for the treatment of cochlear synaptopathy-associated hearing loss and the preservation of cochlear neurons. Correspondingly, OTO-413 has demonstrated benefit in a Phase 1/2 clinical trial in subjects with speech-in-noise hearing impairment across various intelligibility metrics.

Disclosures: C. Fernandez: A. Employment/Salary (full or part-time):; Otonomy, Inc.. **N. Tsivkovskaia:** None. **X. Wang:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **R. Fernandez:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **T. Altmann:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **P. Uribe:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **P.D. Mathur:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **Y. Tsabur:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **S. Siegel:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **S. Szobota:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **A. Stepanenko:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **B. Jacques:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **A.C. Foster:** A. Employment/Salary (full or part-time):; Otonomy, Inc. **F. Piu:** A. Employment/Salary (full or part-time):; Otonomy, Inc..

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.06

Topic: D.06. Auditory & Vestibular Systems

Title: Aav-mediated gene therapy with oto-825 rescues hearing loss and cochlear degeneration in two mouse models of gjb2 congenital hearing loss

Authors: *P. URIBE[1], P. D. MATHUR[1], X. WANG[1], S. SZOBOTA[1], J. BARDEN[1], S. SIEGEL[1], C. L. BARTOLOME[2], D. JARAMILLO[2], A. HARROP-JONES[1], R. FERNANDEZ[1],

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Abstract: Congenital hearing loss is the most common inherited sensory disorder. Of the >100 genes associated with hereditary deafness, most are expressed by mechanosensitive hair cells or their neighboring support cells. The *GJB2* gene encodes the protein connexin26 (CX26) which forms gap junctions across multiple cell types within the cochlea, and mutations in this gene represent the most common cause of genetic hearing loss. Results from mouse and human studies have shown that *GJB2* mutations lead to degeneration of cochlear structures within the organ of Corti. We previously reported a class of novel AAV capsids that efficiently transduce cochlear cell types that natively express CX26 in rodents and nonhuman primates. We then further optimized the AAV construct by packaging the novel capsid, a broad spectrum promoter, and human GJB2 gene elements with and without a Flag tag (OTO-825-Flag and OTO-825, respectively). To test the efficacy of OTO-825 we utilized *in vitro* assays and two different conditional *Gjb2* knockout mouse models (*Cx26* cKO) to assess *in vivo* rescue of *Gjb2* deficiency. In cell-based assays, utilizing HeLa cells that do not natively express CX26, OTO-825 induced expression of CX26 that was correctly trafficked to the cell membrane, evidenced by typical gap junction plaque-like expression. In the same cells, OTO-825 enhanced uptake and intercellular transfer of gap junction-permeant dyes suggesting that the AAV-delivered CX26 forms functional gap junctions. Intracochlear injection of OTO-825-Flag into rodents or nonhuman primates labeled cell types that natively express CX26 in a plaque-like manner consistent with endogenous CX26. To assess the potential for in vivo rescue using OTO-825, two *Cx26* cKO mouse models were generated by crossing $Cx26^{loxp/loxp}$ mice with either a tamoxifen inducible cre mouse line (*Rosa*) or an inner ear-specific constitutive cre mouse line (*P0*). In both models, cre induction caused severe to profound hearing loss. *Cx26* cKO mice also exhibited severe loss of hair cells, a phenotype that is characteristic of *GJB2* mutations in humans. We then performed postnatal intracochlear injection of OTO-825 or vehicle into *Cx26* cKO mice. Compared with vehicle, administration of OTO-825 to *Cx26* cKO mice substantially restored CX26 expression, rescued hair cell and support cell loss, and more importantly demonstrated functional improvement in hearing across multiple frequencies as measured using auditory brainstem responses (ABR). These encouraging results support the further development of OTO-825 as a clinical candidate for the treatment of congenital hearing loss caused by *GJB2* mutations.

Disclosures: P. Uribe: A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **P.D. Mathur:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **X. Wang:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **S. Szobota:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **J. Barden:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **S. Siegel:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **C.L. Bartolome:** A. Employment/Salary (full or part-time):; Applied Genetic Technologies Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Genetic Technologies Corporation. **D. Jaramillo:** A. Employment/Salary (full or part-time):; Applied Genetic Technologies Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Genetic Technologies Corporation. **A. Harrop-Jones:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **R. Fernandez:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **J. Vestal:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **R. Pastenes:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **B.E. Jacques:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **S. Pennock:** A. Employment/Salary (full or part-time):; Applied Genetic Technologies Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Genetic Technologies Corporation. **A. Timmers:** A. Employment/Salary (full or part-time):; Applied Genetic Technologies Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Genetic Technologies Corporation. **F. Piu:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc. **M. Shearman:** A. Employment/Salary (full or part-time):; Applied Genetic Technologies Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Genetic Technologies Corporation. **A.C. Foster:** A. Employment/Salary (full or part-time):; Otonomy, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Otonomy, Inc..

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.07

Topic: D.06. Auditory & Vestibular Systems

Support: NWO Grant #016.Veni.188.049
NIH Grant R01-DC002390
NIH Grant R01-DC013069
NSERC Grant RGPIN: 356026-13

Title: Asymmetry in vestibular afferent responses to transmastoid galvanic vestibular stimulation

Authors: *P. A. FORBES[1], A. KWAN[2], D. E. MITCHELL[3], J.-S. BLOUIN[4], K. E. CULLEN[5]; [1]Erasmus Med. Ctr., Rotterdam, Netherlands; [2]McGill Univ., Montreal, QC, Canada; [3]Dept. de neurosciences, Univ. De Montréal, Montreal, QC, Canada; [4]Univ. of British Columbia, Vancouver, BC, Canada; [5]Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: Vestibular afferent activity, which encodes head movement and orientation, can be modulated by delivering galvanic vestibular stimulation (GVS) through electrodes placed on the mastoids. This virtual signal of head motion has grown as a unique technique to investigate and manage vestibular disorders, and provide artificial sensations of motion for virtual reality applications. Establishing a direct relationship between GVS- and motion-evoked vestibular responses is critical for developing methods that mimic natural motion. Indeed, dynamic models (i.e. transfer functions) of GVS-evoked afferent responses are used to estimate and generate motion equivalent activation of vestibular afferents using GVS. The underlying assumption, however, is that GVS-evoked activity has symmetric effects on afferent firing rates across stimulus polarity. Here we directly investigated whether vestibular semicircular and otolith afferents symmetrically encode opposing polarities of transmastoid GVS. We recorded responses from individual vestibular afferents in macaque monkeys during prolonged (40 s) constant current stimulation using a bilateral bipolar configuration. We found that the onset and offset of constant current GVS evoked significant asymmetries in irregular but not regular afferent responses. While significant asymmetries were seen in both semicircular canal and otolith systems, they were most pronounced for irregular otolith afferents. Similar asymmetries were observed when comparing afferent responses to cathodal and anodal stimulation; the offset of a cathodal stimulus was comparable to the onset of an anodal stimulus, and vice versa. Likewise, afferent response dynamics differed for stochastic GVS stimulation that spanned the physiologically relevant range of the vestibular system (0-25 Hz) and the application of single sinusoidal GVS stimulation over the same range. Using computational methods, we establish that a nonlinear model that separately combines the dynamics of afferent responses to anodal and cathodal stimuli can explain the changes in firing rate evoked by both constant current and stochastic GVS. Finally, and surprisingly, using established models of neural activity to natural motion, we found that in both stimulus directions the sustained firing rate throughout stimulation is encoded as constant rotational and linear acceleration signals for the canals and otoliths, respectively. Taken together, our results reveal the neural substrate underlying asymmetries in

GVS-evoked perceptual and responses, thereby providing new insights in developing physiologically relevant models and advancing the application of GVS.

Disclosures: P.A. Forbes: None. **A. Kwan:** None. **D.E. Mitchell:** None. **J. Blouin:** None. **K.E. Cullen:** None.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.08

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01NS110893
NIH RN01NS092726
NSF GRFP DG31746891

Title: Galvanic stimulation of the vestibular afferent reveals novel ability to produce naturalistic firing patterns via pre-synaptic and axonal modulation

Authors: *C. STEINHARDT[1], G. FRIDMAN[2];
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Abstract: Galvanic vestibular stimulation (GVS) is a common tool for probing computations of the vestibular system and regularly used in the clinic to diagnosis eye movement conditions without requiring physical displacement. An on-going debate surrounds how GVS activates neurons to induce these eye motions, including to what extent it affects afferents of different regularity and via which mechanism it drives changes in firing rate. In previous work, cathodic and anodic direct current (DC) and sinusoidal stimulation were introduced to irregular afferents *in vitro*, revealing afferents are highly sensitive to GVS, smoothly modulate firing rate proportional to GVS amplitude while preserving CV*, and show high pass filtering effects in response to sinusoidal modulation. In this study, we form a cohesive explanation for these effects by systematically refining the Hight and Kalluri (2016) model until a single simulation neuron could replicate the results of all experiments. The original Hight and Kalluri model of the vestibular hair cell-axon complex simulates an axon with realistic channel dynamics receiving stochastic EPSC input. Using this model, we find that when GVS directly affects the axon, it raises or lowers the membrane potential in turn increasing or decreasing the probabilities of EPSCs becoming action potentials. Because the spike timing derives from the timing of EPSCs, GVS preserves firing statistics (CV*) while modulating the firing rate. The other effects can only be explained if GVS triggers two additional natural effects of mechanical perturbation of the hair bundle: hair cell adaptation pathways and the non-quantal effect. When the simulation includes equations so that GVS produces these effects as well, it replicates all experimental findings from

the *in vitro* study. These findings corroborate previous evidence that GVS affects the hair cell and put forward a wholistic model with testable predictions to resolve this debate. More generally, our findings indicate that GVS it has a unique ability to produce axonal, synaptic, and pre-synaptic effects and activate natural pathways. These capabilities position galvanic stimulation as an exciting tool for scientific exploration and clinical interventions in the brain and peripheral nervous system.

Disclosures: C. Steinhardt: None. **G. Fridman:** None.

Poster

P431. Hair Cells and the Periphery: Therapeutic Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P431.09

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC018083-01

Title: The effect of activating a vestibular prosthesis on the activity of units recorded in 8th nerve of monkey.

Authors: L. LING[1], Y. KOJIMA[1], A. NOWACK[1], S. D. NEWLANDS[3], J. O. PHILLIPS[2]; [2]Otolaryngology - HNS, [1]Univ. of Washington, Seattle, WA; [3]Otolaryngology, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Introduction: Vestibular loss is often caused by damage to the sensory periphery. A vestibular prosthesis can theoretically restore function by bypassing the damaged region and activating vestibular afferents directly. This study evaluated how vestibular afferent fibers respond to prosthetic activation.
Methods: We simulated natural vestibular loss in rhesus monkeys by repeated bilateral injection of gentamicin into the middle ear, causing reduction of the passive rotational VOR gain. We recorded from fibers in the 8th nerve to examine how activation of the vestibular prosthesis is reflected in the pattern of discharge. We extracted unit activity from the field potential that follows the stimulus artifact. We estimated the pure field potential for each stimulus condition to be subtracted from the raw signal to reveal the underlying single unit activity.
Results: Many units with regular tonic discharge show no change in activation from any electrode of the vestibular prosthesis at maximal safe currents (without overt spread to facial nerve). Some regular units are driven in a canal specific manner. Many spikes following the stimulus pulse are shifted to be aligned at short latency (0.6-0.8 ms) whereas the rest of the spike train remains unaffected. Typically, spikes are consistently elicited by stimulation from a single canal site, and then less consistently elicited from another canal. Units with irregular tonic discharge display added spikes which are elicited at short latency (0.5-0.9 ms) following

stimulus pulses generated by the implanted device. The stimulus-triggered histogram shows a clear gap following the stimulus artifact. However, the added spikes are not exclusively elicited by electrical stimulation of the canal in the plane of the passive rotational response of the afferent fiber. In addition, we encountered 8th nerve fibers driven inconsistently at short latency, but consistently at synaptic latencies of 1.5-2.0 ms, and which pause during saccades. We hypothesize that these are vestibular efferents.
Conclusion: Multiple types of response are seen in 8th nerve fibers in response to stimulation with a vestibular prosthesis. This variety of discharge may provide a clue to the mechanism underlying afferent activation with such devices.

Disclosures: L. Ling: None. **Y. Kojima:** None. **A. Nowack:** None. **S.D. Newlands:** None. **J.O. Phillips:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.01

Topic: D.06. Auditory & Vestibular Systems

Support: NSF IOS 1656360

Title: Sex differences and the influence of auditory experience on premotor HVC neurons of juvenile zebra finches

Authors: *A. BRUNICK[1], R. BERTRAM[2], F. JOHNSON[3], R. L. HYSON[3];
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Abstract: Juvenile zebra finches undergo auditory learning when exposed to an adult tutor's song during development, though only males will go on to produce a song as adults. The network that modulates vocal output is largely conserved between males and females, though the nuclei tend to be much smaller in females. The cortical premotor nucleus HVC (proper name) projects to two motor regions (robust nucleus of the arcopallium [RA] & Area X), allowing for direct and indirect modulation of vocal motor output, respectively. The influence of these pathways on vocal motor output changes throughout development, in males. HVC is critical for timing of adult song production and is sensitive to auditory experience. The function of HVC in females, particularly during development, is poorly understood. To better understand the differences between male and female HVC, whole cell recordings were obtained from sections of HVC to compare the electrophysiological features of HVC neurons in juvenile males and females raised with or without a tutor, at the time males begin sensorimotor learning (~40 days post hatch). Recorded neurons were filled with biocytin for later confirmation of their location within HVC

and to confirm cell identity when possible. Preliminary results demonstrate that there are sex differences in the spike width and spike accommodation of HVC_X neurons, and these effects seem to be sensitive to auditory experience. Potential group differences in putative HVC_{RA} neurons have also emerged including, but not limited to, changes in spike latencies and voltage sag. The effect of auditory experience on physiology differs between males and females, suggesting that different mechanisms mediate these changes. However, there is more overlap in the features than there are differences, which suggests that HVC neurons have "default" physiological states. These differences due to auditory experience expand upon prior investigation of HVC neuron physiology and demonstrate that there is considerably more heterogeneity within cell groups than expected. This means that any effects of auditory experience are more nuanced than previously thought. These subtle physiological changes could have large impacts on activity propagation within HVC, particularly when examining HVC's microcircuitry.

Disclosures: A. Brunick: None. **R. Bertram:** None. **F. Johnson:** None. **R.L. Hyson:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.02

Topic: D.06. Auditory & Vestibular Systems

Support: NSF Grant 1656360

Title: Vocal-motor network in the female zebra finch

Authors: *D. SHAUGHNESSY[1], R. BERTRAM[1], F. JOHNSON[1], R. L. HYSON[2];
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Abstract: The zebra finch (Taeniopygia guttata) is a songbird whose expertise in vocal learning is studied for insight into how brain & behavior contribute to the learning, encoding, and production of a vocal-motor skill — singing. Zebra finch song production is sexually dimorphic, where only male zebra finches learn to produce song although both sexes form an auditory memory of adult tutor song during a developmental critical period. This offers a within-species contrast between subjects who experience auditory learning + motor learning (males) and subjects who experience only auditory learning (females) in the song-learning context. One hypothesis for why only males sing is that only males possess the requisite neural circuitry for song production. This hypothesis is supported by a handful of early zebra finch neuroanatomy papers which reported that the network of discrete brain regions linked to song in male brains were mostly absent in females. Previously we used a novel surgical visualization technique to target tracer injections into female vocal-premotor cortex (**HVC**, proper name) - demonstrating

formerly uncharted male-typical connections to a range of other song network nodes. Notably, adult female HVC robustly innervates female vocal-motor cortex (**RA**, robust nucleus of the arcopallium), a connection which is integral to learned song production in male zebra finches. In males, RA integrates cortical pre-motor input from HVC and LMAN (lateral magnocellular nucleus of the anterior nidopallium), and projects to vocal-motor control regions of the brainstem. In the present study we continue mapping female vocal-motor circuitry by examining the anterograde and retrograde connectivity of female RA. Here we inject bidirectional fluorescent dye into female RA and verify injection sites histologically. In RA-injected female brains we find male-typical retrograde connectivity to HVC and LMAN and male-similar anterograde projection in the vocal brainstem. Our findings demonstrate the potential of the female zebra finch brain to advance our understanding of the division of sensory & sensorimotor learning within the song network.

Disclosures: D. Shaughnessy: None. **R. Bertram:** None. **F. Johnson:** None. **R.L. Hyson:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIH IOS Grant 1656360

Title: Automated classification of neurons based on electrophysiological features

Authors: *P. EASTHAM, A. BRUNICK, R. HYSON, F. JOHNSON, R. BERTRAM; Florida State Univ. Program In Neurosci., Tallahassee, FL

Abstract: Electrophysiology experiments are often conducted in an effort to categorize neurons into different classes based on exhibited features. A primary difficulty in categorizing neurons is the subjective choices of the experimenter which, to the detriment of experimental rigor, makes reproducing or comparing classifications across labs difficult. Additionally, there is no measure for choosing a representative neuron from a given class which, for example, can be used in a data visualization in a manuscript. We describe a novel method for the automated classification of neuronal phenotypes based on electrophysiological data. This method relies on generating network graphs whose nodes are biological neurons and weighted edges represent the “degree of similarity” between neurons. Using existing graph theory and optimization algorithms leads to the automatic classification of neuronal phenotype based on features and, furthermore, allows selection of a representative neuron for each class. As a demonstration, we apply this method to classify physiological properties of Zebra Finch HVC neurons. This method is accessible for

neuroscience researchers and will aid in the reproducibility of neuronal phenotype classification. In particular, it provides a rigorous and objective method for investigators to define the representative case when presenting physiological data.

Disclosures: P. Eastham: None. **A. Brunick:** None. **R. Hyson:** None. **F. Johnson:** None. **R. Bertram:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.04

Topic: D.06. Auditory & Vestibular Systems

Support: BRAIN Initiative NCS-FO 1734744
NIH Grant 5T32DC000023-35
ONR N00014-17-1-2736
AFOSR FA9550-14-1-0398NIFTI

Title: Just around the corner: Acoustic mirroring enables echolocating bats to detect hidden targets

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Abstract: The ability to detect salient targets and segregate them from background clutter is a key function of sensory systems. The presence of clutter stimuli typically degrades sensory performance in a variety of detection tasks. The echolocating bat Eptesicus fuscus is an ideal model for investigating the influence of clutter on target detection, as they must navigate through dense foliage at high speed, while simultaneously tracking small moving prey. This difficult task requires bats to reliably detect and track objects in the environment despite changes in distance, angle of view, and interference from background clutter echoes, which obscure targets from sonar “view” and creates physical barriers bats must navigate around. However, recent studies reveal that environmental clutter echoes may improve performance under some conditions. Previous neurophysiological recordings in the bat inferior colliculus indicate that acoustic scattering caused by physical clutter from foliage can improve target discrimination when the separation between target and clutter is ~20 cm. These findings suggest the possibility that acoustic reflections from environmental clutter may provide additional “glimpses” of targets, and under the correct conditions, improve target discrimination. Here, we test the ability of echolocating bats to make use of acoustic mirrors from clutter objects for target detection using a yes/no behavioral assay inspired by Non-Line of Sight Imaging experiments. Much like technology employed by machine imaging, robotics, and self-driving automobiles, we test the

potential for bats to “see around corners” using only echoes reflected from objects in the environment to locate a target hidden behind an occluder.

Disclosures: K.M. Allen: None. **C. Farid:** None. **M. Fu:** None. **C.F. Moss:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.05

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R34NS118462

Title: Two-photon calcium imaging reveals functional microarchitecture for auditory processing in the inferior colliculus of the awake bat

Authors: *J. LAWLOR BLONDEL[1], Y. SUEOKA[2], P. GUTRUF[3], M. J. WOHLGEMUTH[4], C. F. MOSS[1], K. KUCHIBHOTLA[1];
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Abstract: Navigating our everyday world requires parsing relevant information from constantly evolving sensory flows. How the brain processes and sorts sensory inputs is a central ongoing question in systems neuroscience. Here, we take advantage of a model long-studied for its expert auditory sensing of the world: the echolocating bat. The echolocating bat produces ultrasonic vocalizations and listens to returning echoes to determine the identity and location of objects in the environment. As such, the bat constructs a rich representation of the external environment primarily using the auditory modality, making it a powerful model to study how the brain represents natural acoustic signals. While traditional multi-channel electrophysiology techniques have provided key insights into network-level activity, they are limited in their ability to reveal micro-functional architecture and cell-type specific activity. We developed two-photon calcium imaging in the awake big brown bat, *Eptesicus fuscus*, to assay the activity of a population of neurons with cellular and sub-cellular resolution. We expressed GCaMP6f in the excitatory population of the Inferior Colliculus, a central auditory hub, while using a head-fixation and thinned-skull surgical approach to longitudinally monitor the same local population over days to weeks. We assessed functional auditory properties of thousands of neurons in awake, passively listening bats (n=3) by presenting pure tones, white noise, frequency sweeps, echolocation and social calls, and other stimuli. We parametrically controlled features including the duration and delay of relevant stimuli. In preliminary analyses, we observe a novel, fine-scaled tonopy in the superficial layers of the IC. In addition, presentation of ‘prey capture’ echolocation sequences

varying in their spectral content (‘natural’ vs ‘artificial’) elicited a population rotational dynamic reflecting the temporal structure of the call sequence, while manifolds separate the spectral information. In the longer-term, we will use these studies as an entry point to understand the neural basis of active sensing in the context of ethologically-relevant behaviors by combining cell-type specific calcium imaging with wireless optogenetics.

Disclosures: J. Lawlor Blondel: None. **Y. Sueoka:** None. **P. Gutruf:** None. **M.J. Wohlgemuth:** None. **C.F. Moss:** None. **K. Kuchibhotla:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.06

Topic: D.06. Auditory & Vestibular Systems

Title: Vocal perceptual category boundaries are modified by maternal experience in female mice

Authors: *L. SHEN, S. D. SHEA;
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Abstract: Only a fraction of sounds an organism encounters are behaviorally significant. One example is vocalizations, which occur against a backdrop of sounds with overlapping features, while exhibiting substantial variability. This presents two competing challenges: detection must be selective enough to minimize false alarms while also being tolerant to natural variation. Here we use a vocally-motivated pup retrieval behavior to reveal how the brain adjusts and enforces perceptual boundaries between vocal signals and other sounds. When pups are separated from their nest, they emit an ultrasonic vocalization (USV) that maternally-experienced mice can use to locate and retrieve the pups back to the nest. We have developed a high-throughput, quantitative behavioral paradigm where nulliparous female mice are water-deprived and trained in a probabilistic go/no-go task. They are conditioned to lick a water spout in response to playback of a USV, and to withhold licking in response to a synthetic tone. Once stable performance (>80%) is achieved, the mice are separated into two groups: mice that are exposed to pups and gain maternal experience (‘surrogates’), and mice that have never been exposed to pups (‘naïves’). We next present a set of novel sounds to the mouse as infrequent and unrewarded catch trials (including bandwidth-limited noise and pitch-shifted USVs), and we use licks to the novel stimuli as a measure of perceived similarity to natural USVs. These infrequent stimuli are not rewarded, and USVs are rewarded on 80% of trials. The catch trials demonstrate that mice response rates vary systematically with the catch trials stimuli similarity to natural USVs, a result supported by a freely moving behavioral task where mothers are timed in how quickly they approach a speaker playing the catch trial sounds. Furthermore, we find that responses to the synthetic sounds show evidence of learning between initial and late trials that

differs in naïves and surrogates. Initial responses of surrogates towards broadband sounds in the range of USVs are similar to those of the Go-Cue, while naïve mice tend to initially have lower response rates to those same sounds. Late responses of surrogate mice toward those same sounds were generally lower than those of the naïve mice. Finally, electrophysiological data in the primary auditory cortex done before and after behavioral training suggests that those neurons adjust their firing rate following both pup exposure and task learning. Taken together, our results suggest that vocal perceptual filters are optimized during maternal experience, and will provide insight into how categorical perception contributes to speech processing and learning.

Disclosures: L. Shen: None. **S.D. Shea:** None.

Poster

P435. Auditory Processing: Vocalizations and Natural Sounds II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P435.07

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC017906
NIH Grant DC013826
NIH Grant MH117778
George Barth Geller Fund

Title: Inferior colliculus neurons respond to individual syllables of mouse ultrasonic vocalizations with high specificity

Authors: *B. J. SLATER, R. D. MOONEY;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: The fidelity of auditory stimulus representation varies markedly from the periphery to the cortex. The coding behavior of earlier nuclei is characterized by neuronal responses statistically similar to a given stimulus progressing to higher order nuclei with sparse representation for even complex stimuli, such as vocalizations. The transformation of high to low statistical fidelity in the auditory system is most often characterized in reference to pure tone stimuli. However, it is unclear how these pure tone responses relate to the neural coding of vocalizations, which are by spectrotemporally more complex and dynamic than tones. Neurons in the inferior colliculus (IC) can exhibit complex and specific responses to acoustic signals as a function of their timing, intensity, and frequency, but how this complexity relates to natural sound stimuli remains unclear. Using extracellular recordings in the IC of awake head-fixed mice, we examined neural responses to both pure tones as well as playback of ultrasonic vocalizations (USVs) previously recorded during courtship behavior. As expected, most IC neurons responded to pure tones in a frequency-dependent manner, the greatest response being

referred to as a neuron's best frequency. We also found a majority of IC neurons responded to playback of USVs with high temporal precision, and often only to a single syllable in the USV bout. Curiously, we did not find a good correlation between an IC neuron's best frequency and mean frequency of the USV syllable(s) that it responded to. We also found that while some neurons responded to a number of different syllables, there were no units that were responsive to all syllables within bout. This indicates that there is particular specificity of IC neurons to individual syllables within the animal's vocal repertoire.

Disclosures: B.J. Slater: None. **R.D. Mooney:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC014101
NIH Grant R01NS116598
NIH Grant R01MH122478
NIH Grant R01EY025174
PBBR Breakthrough Fund
Coleman Memorial Fund
Hearing Research Institute

Title: Cell type specific short-term plasticity of sound offset responses in the auditory cortex

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Abstract: The neural response to sound is dependent on stimulus history, with sound-evoked responses subject to various forms of short-term plasticity (STP). In addition to spiking in response to sound onsets, many neurons in the central auditory system fire action potentials in response to the cessation of a sound (so-called "offset responses"). Sound offset responses are essential for aspects of sound processing including the encoding of sound duration and the discrimination of up versus down frequency-modulated sweeps, which in turn are essential for the understanding of continuous sounds such as speech. Whether offset responses are also subject to STP is currently unknown. We here study the history-dependence of sound offset responses among units in the primary auditory cortex (A1). Inhibitory interneurons in A1 are also subject to STP, and their activity in turn shapes the temporal response profile of excitatory neurons. We therefore also measured offset STP in PV- and SST-tagged inhibitory neurons. We presented awake mice with a series of repeating noise bursts, recorded through the layers of

cortex with a silicon probe, and measured dynamics of spiking response magnitudes. Single units responded to sound onsets, offsets, and/or both, with purely offset responsive neurons far less common than purely onset responsive neurons. Onset responses mainly adapted over repeated sound stimuli, with a smaller proportion of units showing either no change or facilitation. In contrast, sound offset responses were less likely to adapt and more likely to either remain stable or facilitate. Among units which showed both onset and offset responses, onset response plasticity was not predictive of offset response plasticity, and vice versa. PV-tagged interneurons typically showed both onset and offset responses, with their onset responses predominantly adaptive and their offset responses more likely to be either stable or facilitating. SST-tagged neurons typically showed onset responses but not offset responses, with their onset responses more likely to facilitate than those of broad-spiking and PV-tagged units. This study reveals that offset responses in A1 are subject to short-term plasticity, with different cell types in A1 showing varying amounts of onset and offset adaptation and facilitation. The discovery of offset STP presented in this study increases our understanding of the auditory system's response to continuous sound, and provides a foundation for future research into the function of offset responses in a brain where response magnitudes are infrequently static over time.

Disclosures: T.R. Olsen: None. **A.R. Hasenstaub:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC015527
NIH NIDCD R01DC014479
NIH NINDS R01NS113241
NIH NIDCD F31DC016524

Title: Cortical efficient coding dynamics shape behavioral performance

Authors: *C. F. ANGELONI[1], W. F. MLYNARSKI[2], E. PIASINI[1], A. M. WILLIAMS[1], K. C. WOOD[1], L. GARAMI[1], A. M. HERMUNDSTAD[3], M. N. GEFFEN[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Brain and Cognitive Sci., Inst. of Sci. and Technol. Austria, Klosterneuburg, Austria; [3]Janelia Res. Campus, Ashburn, VA

Abstract: The efficient coding hypothesis postulates that neurons shape their response properties to match their dynamic range to the statistics of incoming signals. However, whether and how the dynamics of efficient neuronal adaptation inform behavior has not been directly shown. Here, we trained mice to detect a target presented in noise shortly after a change in the noise contrast.

The observed changes in cortical gain and detection behavior followed the predictions of a normative model of efficient cortical sound processing; specifically, target detection and sensitivity improved in low contrast relative to high contrast noise. Furthermore, the time course of cortical gain and target detectability varied depending on contrast, decreasing rapidly after a transition to high contrast, and increasing at a slower rate after a transition to low contrast. We found that the auditory cortex was required for detection of targets in noise and neuronal responses exhibited the predicted patterns of target detectability. Furthermore, the magnitude of gain modulation in cortical neurons predicted individual differences in behavioral performance. Combined, our results demonstrate that efficient neural codes in auditory cortex directly influence perceptual behavior.

Disclosures: C.F. Angeloni: None. **W.F. Mlynarski:** None. **E. Piasini:** None. **A.M. Williams:** None. **K.C. Wood:** None. **L. Garami:** None. **A.M. Hermundstad:** None. **M.N. Geffen:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01DC018353
NIDCD F32DC018211
Bertarelli Foundation
Nancy Lurie Marks Family Foundation
Natural Sciences and Engineering Research Council of Canada
Fonds de Recherche du Québec Nature et Technologies

Title: Function of cortical NDNF interneurons in sound frequency discrimination

Authors: *M. E. THOMAS[1], C. G. SWEENEY[1], E. Y. YU[2], K. SMITH[1], W. REID[3], A. E. TAKESIAN[1];
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Abstract: Sound frequency discrimination, the ability to distinguish between pure tones of different frequencies, underlies both speech and music perception. A growing understanding of auditory cortical interneuron circuits has highlighted the role of specific inhibitory interneurons in shaping frequency tuning and discrimination acuity. Here, we focus on a recently identified class of interneurons that express neuron-derived neurotrophic factor (NDNF) and reside primarily within neocortical layer 1. In auditory cortex, NDNF interneurons integrate direct sensory inputs from the auditory thalamus and long-range neuromodulatory signals from diverse

brain regions. In turn, these interneurons powerfully inhibit the distal dendrites of excitatory neurons while simultaneously disinhibiting their somata via deeper projections to parvalbumin-positive interneurons. While this circuitry suggests that NDNF interneurons are poised to shape excitatory neuron tuning, little is known about their function in sound processing. Thus, the present research aims to investigate the function of NDNF interneurons in frequency tuning of neurons in primary auditory cortex and behavioral frequency discrimination in mice. Using in vivo two-photon calcium imaging, we first characterized the tuning properties of NDNF interneurons in response to pure tones of varying frequencies and intensities. We found that a subset of NDNF interneurons exhibit robust frequency and intensity tuning comparable to excitatory neurons in layer 2/3. We next developed a behavioral frequency discrimination paradigm in which mice distinguished trains of repeating pure tones of a single frequency from trains of two alternating tones of different frequencies. Mice readily learned this task within two weeks, allowing us to establish reliable frequency discrimination thresholds by varying the frequency of the alternating tone. We subsequently recorded the responses of both NDNF interneurons and layer 2/3 excitatory neurons to the training stimuli under both passive and behaving contexts. We identified individual neurons in both neuron classes capable of discriminating between repeating or alternating tones, and future analyses will assess the population dynamics associated with behavioral discrimination. Finally, ongoing experiments using chemogenetic silencing of NDNF interneurons will demonstrate the necessity of their activity for task performance. This work will help establish the function of NDNF interneurons in frequency discrimination and further elucidate the contribution of specific inhibitory cortical circuits to sound processing.

Disclosures: M.E. Thomas: None. **C.G. Sweeney:** None. **E.Y. Yu:** None. **K. Smith:** None. **W. Reid:** None. **A.E. Takesian:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIMH F32MH127788
NIH NIDCD R01DC015527
NIH NIDCD R01DC014479
NIH NINDS R01NS113241
Kaufman Foundation New Initiative Research Grant #KA2020-114796

Title: Olfactory modulation of auditory cortical responses to sound

Authors: *N. W. VOGLER, T. LING, A. C. VIRKLER, V. Y. TU, J. A. GOTTFRIED, M. N. GEFFEN;
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Abstract: In complex environments, the brain must integrate information from multiple sensory modalities, including the auditory and olfactory systems, for perception and behavior. However, despite the importance of multisensory integration, there is a critical gap in our understanding of how the brain integrates auditory and olfactory stimuli. Here, we investigated the mechanisms underlying auditory-olfactory integration using electrophysiology, anatomy, and behavior. First, we tested whether and how odor stimuli modulate auditory cortical neurons' responses to sound in awake mice. We developed an experimental system for delivering combinations of auditory and olfactory stimuli, and we collected *in vivo* electrophysiological recordings from the auditory cortex (ACx) in response to sounds, in the presence or absence of an odor. Our results demonstrate that ACx neurons exhibit greater firing rates in response to an odor stimulus (odor+air) compared to air alone. Furthermore, the presence of odor changes the gain of sound responses. Together, these results demonstrate that ACx neurons are odor-responsive, and that odors modulate sound responses in awake mice. Next, we used viral tracing strategies to investigate the anatomical circuits underlying auditory-olfactory integration. Our results demonstrate direct inputs to the ACx from the piriform cortex (PCx), suggesting a possible substrate for olfactory integration in ACx. Finally, behavioral experiments are investigating how odor stimuli influence sound detection. Combined, our results point to ACx as an important area for auditory-olfactory integration.

Disclosures: N.W. Vogler: None. **T. Ling:** None. **A.C. Virkler:** None. **V.Y. Tu:** None. **J.A. Gottfried:** None. **M.N. Geffen:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.05

Topic: D.06. Auditory & Vestibular Systems

Support: PEW Biomedical Program for Latin American Fellows
Fonds de Recherche du Québec Nature et Technologies
Natural Sciences and Engineering Research Council of Canada
NIDCD F32DC018211
NIDCD R01DC018353
Bertarelli Foundation
Nancy Lurie Marks Family Foundation

Title: Recurrent network activity in layer 1 interneurons of the mouse primary auditory cortex

Authors: *L. G. VATTINO[1], M. E. THOMAS[1], C. G. SWEENEY[1], R. BRITO[2], C. P. MACGREGOR[3], A. E. TAKESIAN[1];
[1]Massachusetts Eye and Ear - Harvard Med. Sch., Boston, MA; [2]Massachusetts Eye and Ear - Speech and Hearing Biosci. and Technology, Div. of Med. Sciences, Harvard Med. Sch., Boston, MA; [3]Massachusetts Eye and Ear, Boston, MA

Abstract: Inhibitory interneurons in neocortical layer 1 (L1) convey behaviorally relevant information by integrating sensory-driven inputs with neuromodulatory signals. Their activity is known to regulate moment-to-moment encoding of the sensory environment in a context-dependent manner and can drive cortical plasticity mechanisms, both during postnatal development and adulthood. We and others have shown that these interneurons are heterogenous and can be subdivided into two major classes defined by the expression of either neuron-derived neurotrophic factor (NDNF) or vasoactive intestinal peptide (VIP). It has been previously demonstrated that L1 interneurons make recurrent synaptic contacts and are also connected electrically through gap junctions, suggesting that they might form coordinated inhibitory networks. However, the connectivity patterns of specific L1 interneuron subtypes and the *in vivo* functional implications remain unclear. We performed fluorescence-guided whole-cell electrophysiology in slices of the mouse primary auditory cortex (A1) while optogenetically activating VIP or NDNF interneurons. We found that $GABA_A$-mediated synaptic connections between NDNF interneurons were significantly stronger than those between VIP interneurons or other L1 interneurons, suggesting a robust NDNF recurrent inhibitory network. Next, we performed two-photon calcium imaging in A1 of awake, behaving mice to monitor the spontaneous and sound-driven activity of networks of VIP and NDNF interneurons. These results will reveal how behavioral context impacts the *in vivo* coordinated activity of these L1 inhibitory networks. Together, our findings suggest that distinct connectivity patterns among NDNF and VIP interneurons may underlie specialized functions in sensory encoding and cortical plasticity.

Disclosures: L.G. Vattino: None. **M.E. Thomas:** None. **C.G. Sweeney:** None. **C.P. MacGregor:** None. **A.E. Takesian:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC015527
NIH NIDCD R01DC014479
NIH NINDS R01NS113241

NIH NIDA DA044205
NIH NIDA DA049545
NIH NIAAA U01 AA025931
NIH F32 MH120890

Title: Cortico-fugal regulation of predictive coding

Authors: *A. M. H. LESICKO[1], C. F. ANGELONI[1], J. M. BLACKWELL[2], M. DE BIASI[1], M. N. GEFFEN[1];
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Abstract: Sensory cues are differentially encoded depending on the contextual stream in which they are embedded. This adaptation can be understood through a predictive coding framework, in which responses to predictable stimuli become attenuated, in a process known as repetition suppression, while unexpected cues elicit a prediction error response. In the auditory system, prediction error first appears at the level of the auditory midbrain, or inferior colliculus (IC), and is most prominent in the auditory cortex (AC), suggesting that these regions engage in hierarchical predictive coding. To determine whether descending connections from AC to IC carry top-down predictions, we selectively suppressed the cortico-collicular (CC) pathway while recording responses to predictable and unpredictable stimuli in IC.
To selectively target auditory CC cells, we made bilateral injections of a retro AAV-Cre construct in IC, while injecting an AAV-Flex-ArchT construct in AC. We performed extracellular recordings in IC in awake mice while oddball stimuli, consisting of 90% repeated, or "standard" tones, and 10% deviant tones, were played to the contralateral ear. An additional control sequence that was free of repetition effects and contained no statistical deviants was used to compute prediction error and repetition suppression metrics. We suppressed CC cells on a fraction of trials, and quantified differences in predictive coding metrics for laser on and off conditions.
We found that suppression of the CC pathway led to a decrease in prediction error but did not affect repetition suppression, suggesting that this metric may reflect fatigue of bottom-up sensory inputs. We also discovered populations of IC neurons that exhibit repetition enhancement, an increase in firing with stimulus repetition, and error suppression, a stronger response to a tone in a predictable rather than unpredictable context. CC suppression led to a decrease in repetition enhancement and a reduction in error suppression. Neurons in IC responded more similarly to each context in the absence of cortical input, suggesting that AC provides cues about the statistical context of sound to subcortical brain regions.

Disclosures: A.M.H. Lesicko: None. **C.F. Angeloni:** None. **J.M. Blackwell:** None. **M. De Biasi:** None. **M.N. Geffen:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.07

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC015527
NIH NIDCD R01DC014479
NIH NINDS R01NS113241

Title: Differential function of distinct inhibitory neuronal types in cortical networks

Authors: *M. TOBIN[1], J. SHETH[1], K. WOOD[2], M. N. GEFFEN[1];
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Abstract: Cortical neuronal networks are comprised of multiple types of excitatory and inhibitory neurons, which perform specific computations. In the auditory cortex (AC), excitatory neurons receive the sound input and transmit the processed signal to other areas, whereas the inhibitory neurons dynamically help shape the response of the excitatory neurons. Different inhibitory neurons differentially affect sound processing in excitatory neurons, yet how they affect network dynamics remains poorly understood. To establish the role of different interneurons within the cortical network, we stimulated specific interneuron subpopulations, while imaging the network responses to sounds in AC of awake, head-fixed mice. We monitored the activity of populations of hundreds of neurons using two-photon calcium imaging and simultaneously increased the activity of an interneuron subpopulation using optogenetic stimulation. We focused on vasoactive intestinal peptide-positive (VIP) and somatostatin-positive (SST) inhibitory neurons, which have been found to shape cortical responses in a context-dependent fashion (Natan et al, 2015; Pi et al, 2013). Responses of cells activated by sound were enhanced when VIPs were activated, but reduced upon activation of SSTs. The timing of the responses of sound-activated cells did not change with VIP activation, whereas SST activation slowed down their responses. Sound elicited an increase in response in different populations of neurons at different levels of interneuron activation, with more overlap between these populations with VIP than with SST activation. By fitting rate-level functions of single neurons by either a sigmoid (monotonic) or Gaussian (non-monotonic) function, we observed that SST activation displaced the rate-level functions of monotonic cells to higher sound amplitudes and reduced the maximum response in non-monotonic cells. VIP activation led to an upward shift of the rate-level functions to higher responses for monotonic cells and to an increase in the mean and variance for non-monotonic cells. At the population level, we computed measures of detectability and discrimination across neural populations. SST activation decreased population detectability of sounds and increased discriminability between sounds. By contrast, VIP activation increased population sound detectability and decreased discriminability between sounds. We conclude that the activation of VIP or SST interneurons leads to opposite effects on the response of the neural networks to sounds in AC. Our results suggest that the VIP and SST neurons are likely differentially recruited in discrimination and detection behaviors.

Disclosures: M. Tobin: None. **J. Sheth:** None. **K. Wood:** None. **M.N. Geffen:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.08

Topic: D.06. Auditory & Vestibular Systems

Support: ONR grant #ONRN00014-17-1-2736

Title: High call repetition rate enhances objects representation in the bat primary auditory cortex

Authors: *S. MACIAS, K. BAKSHI, M. SMOTHERMAN;
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Abstract: The temporal order and proximity of sounds influence both their perception and neuronal responses. Here, we investigated how internal representation of target's surface in the primary auditory cortex (A1) of bats is affected by the high pulse repetition rates used during echolocation. We recorded the responses of 165 neurons from the A1 of four passive listening Mexican free-tailed bats, *Tadarida brasiliensis*, while stimulating with sequences of pulses and echoes at different repetition rates recorded from surfaces with different roughness. Pulse-echo combinations repeated at 12 and 15 Hz produced a significant reduction in the neuronal response. This decrease in the neurons number of spikes came along with an increase in the response temporal precision and the amount of information carried by spike times across time. In addition, it contributed to neuronal synchronization patterns with a higher signal to noise ratio. We propose that the primary auditory cortex in the bat uses this reduction in the neuronal response as leverage to improve the ability of discriminate objects with different shape and structure.

Disclosures: S. Macias: None. **K. Bakshi:** None. **M. Smotherman:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.09

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01-DC010813

Title: Teasing apart the influences of neural fluctuations and off-CF inhibition in the inferior colliculus using wideband tone-in-noise stimuli

Authors: *J. FRITZINGER, L. H. CARNEY;
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Abstract: There are many proposed mechanisms in the auditory periphery and midbrain for encoding of complex sounds. We hypothesize that comparison of responses to diotic and contralateral wideband tone-in-noise (WB-TIN) stimuli in the inferior colliculus (IC) can differentiate between mechanisms. Two mechanisms of interest are capture and off-characteristic frequency (CF) inhibition. Capture occurs when a tone near the CF of an inner hair cell (IHC) saturates its response, reducing the amplitude of neural fluctuations, the low-frequency changes in auditory-nerve firing rate that arise from nonlinear peripheral filtering of complex sounds. IC neurons are sensitive to amplitude modulation and are hypothesized to respond to neural fluctuations with enhanced or suppressed rates. Other studies have suggested a need for off-CF inhibitory inputs to IC cells to explain sensitivity to frequency sweep direction and psychophysical forward masking. Often the ipsilateral projection to the IC is inhibitory, whereas the contralateral projection is excitatory. We hypothesize that off-CF inhibition will be stronger in response to diotic WB-TIN and that the effect of capture will be similar for diotic and contralateral stimuli. Extracellular single-unit responses were recorded in the IC of two female Dutch-belted rabbits using tetrodes. The WB-TIN stimuli were four-octave noise bands centered on CF with an added tone that varied in frequency across three octaves centered on CF. Diotic or contralateral stimuli were used to investigate peripheral and brainstem contributions to IC responses. In preliminary results, diotic responses to WB-TIN often had inhibitory regions flanking an excitatory region near CF, consistent with off-CF inhibition. Contralateral WB-TIN responses had less sideband inhibition compared to diotic responses, consistent with off-CF inhibitory inputs that arise from ipsilaterally driven inputs to the IC. A current computational IC model for amplitude-modulation tuning, based on same-frequency inhibition and excitation, does not predict the responses to WB-TIN. We are testing an updated model framework with off-CF inhibition. The role of capture in IC responses to WB-TIN can also be investigated using inputs arising from our peripheral model, which features IHC saturation. Responses to diotic WB-TIN indicate the presence of off-CF inhibitory inputs to the IC. Contralateral results show weaker off-CF inhibition in some neurons, indicating ipsilateral inputs to the IC may contain off-CF inhibition. Models that feature different encoding mechanisms will be used to further explore the differences between diotic and contralateral WB-TIN results.

Disclosures: J. Fritzinger: None. **L.H. Carney:** None.

Poster

P443. Auditory Processing: Temporal, Frequency, and Spectral Processing II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P443.10

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant P01-AG055365
NIH Grant R01-DC014085

Title: The effects of aging on the cortical representation of continuous speech

Authors: *I. M. D. KARUNATHILAKE[1], J. L. DUNLAP[2], J. PERERA[2], A. PRESACCO[4], L. DECRUY[3], S. ANDERSON[2], S. E. KUCHINSKY[5], J. Z. SIMON[3];
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Abstract: Selectively attending to speech in noisy environment is crucial in day-to-day interactions, and this becomes more challenging with aging. Neural mechanisms utilized in selective attention and speech perception with age-related differences are not well understood. In this study using magnetoencephalography (MEG), we investigate how continuous speech is perceived and represented in the cortex while attending to one speaker and ignoring the other, at different signal-to-noise ratios. We recorded MEG data from 35 subjects (18 younger, 17 older) with clinically normal hearing. The low frequency (1-10 Hz) neural responses that track the low frequency speech stimulus envelope, both attended and unattended, were investigated using temporal response functions (TRFs) and envelope reconstruction. Consistent with previous findings, older adults exhibit exaggerated speech envelope representation in all SNR conditions and systematic differences between stimulus reconstruction accuracies in attended vs unattended as well as in different SNR conditions. Integration window analysis shows that over-representation starts as early as ~70 ms and older adults' responses need a much longer integration time window (~200 ms) for better reconstruction of the speech envelope. TRF analysis produces three prominent peaks, $M50_{TRF}$, $M100_{TRF}$ and $M200_{TRF,}$ representing distinct auditory processing stages, at corresponding early, middle and late latencies respectively. Older adults exhibit stronger TRF peak amplitudes at all three latencies compared to younger, possibly due to several mechanisms. The $M50_{TRF}$ peak is relatively early in older adults, suggesting speech perception is altered even at early processing stages. In contrast, in older adults the $M200_{TRF}$ is delayed, and its amplitude negatively correlates with its latency, suggesting there are other neural mechanisms contributing to $M200_{TRF}$ modulation. Furthermore, only in older adults, the $M100_{TRF}$ and $M200_{TRF}$ peak amplitude increase and decrease respectively with task difficulty. In both groups all TRF peaks are delayed as the task becomes harder and this effect is larger for older adults, suggesting additional cortical processing is utilized with the task difficulty. Together these results suggest a possible temporal processing deficit and partial compensation in older adults' cortex, and highlights the importance of cortical processing around 200 ms that may compensate for the impaired speech perception.
Disclaimer: The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of Army/ Navy/ Air Force, Department of Defense, or U.S. Government.

Disclosures: I.M.D. Karunathilake: None. **J.L. Dunlap:** None. **J. Perera:** None. **A. Presacco:** None. **L. Decruy:** None. **S. Anderson:** None. **S.E. Kuchinsky:** None. **J.Z. Simon:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.01

Topic: D.07. Vision

Support: NIH NEI R01 EY025219

Title: Evaluating the sensitivity of Functional Ultrasound Imaging for 3D retinotopy in ferret primary visual cortex

Authors: *W. HU[1], M. DOYLEY[1], F. BRIGGS[2];
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Abstract: Functional Ultrasound Imaging (fUS), developed in 2011 [1], has quickly become an important tool in neuroscience research. Compared to traditional imaging methods like functional Magnetic Resonance Imaging (fMRI), fUS provides high spatial and temporal resolution (<0.1mm, >10Hz) with good penetration depth (>15mm). The utility of fUS in measuring the activity of small populations of neurons has not yet been fully determined, however. Here, we examine the ability of fUS to localize and differentiate the representations of visual stimuli placed at different locations within the visual field. Importantly, we reconstruct the retinotopy of the primary visual cortex (V1) of ferrets in a three-dimensional (3D) view. fUS was performed through a craniotomy, allowing imaging access to both V1 and the LGN, with a Vantage 256 scanner (Verasonics, Inc., Kirkland, WA, USA) with an 18 MHz L22-14vX transducer. Responses to drifting gratings placed at distinct positions throughout the field of view were acquired in a series of parallel para-sagittal imaging planes. To generate a ground truth retinotopic map of ferret V1, we combined retinotopic information from electrophysiological recordings [2] with an MRI-based ferret brain atlas template [3] to form a 3D retinotopic model of V1. We then stacked our fUS images to form an fUS volume and registered the fUS volume to the brain atlas template. We find good agreement between the fUS data and the 3D retinotopic model. Our objective is to further quantify the spatial resolution of fUS using an intersectional approach combining optogenetics with fUS imaging and the same 3D reconstruction and modeling pipeline.
Reference:
1. Macé, Emilie, et al. "Functional ultrasound imaging of the brain." Nature methods 8.8 (2011): 662-664.
2. Law, Margaret I., Kathleen R. Zahs, and Michael P. Stryker. "Organization of primary visual cortex (area 17) in the ferret." Journal of Comparative Neurology 278.2 (1988): 157-180.

3. Hutchinson, Elizabeth B., et al. "Population based MRI and DTI templates of the adult ferret brain and tools for voxelwise analysis." Neuroimage 152 (2017): 575-589.

Disclosures: W. Hu: None. **M. Doyley:** None. **F. Briggs:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.02

Topic: D.07. Vision

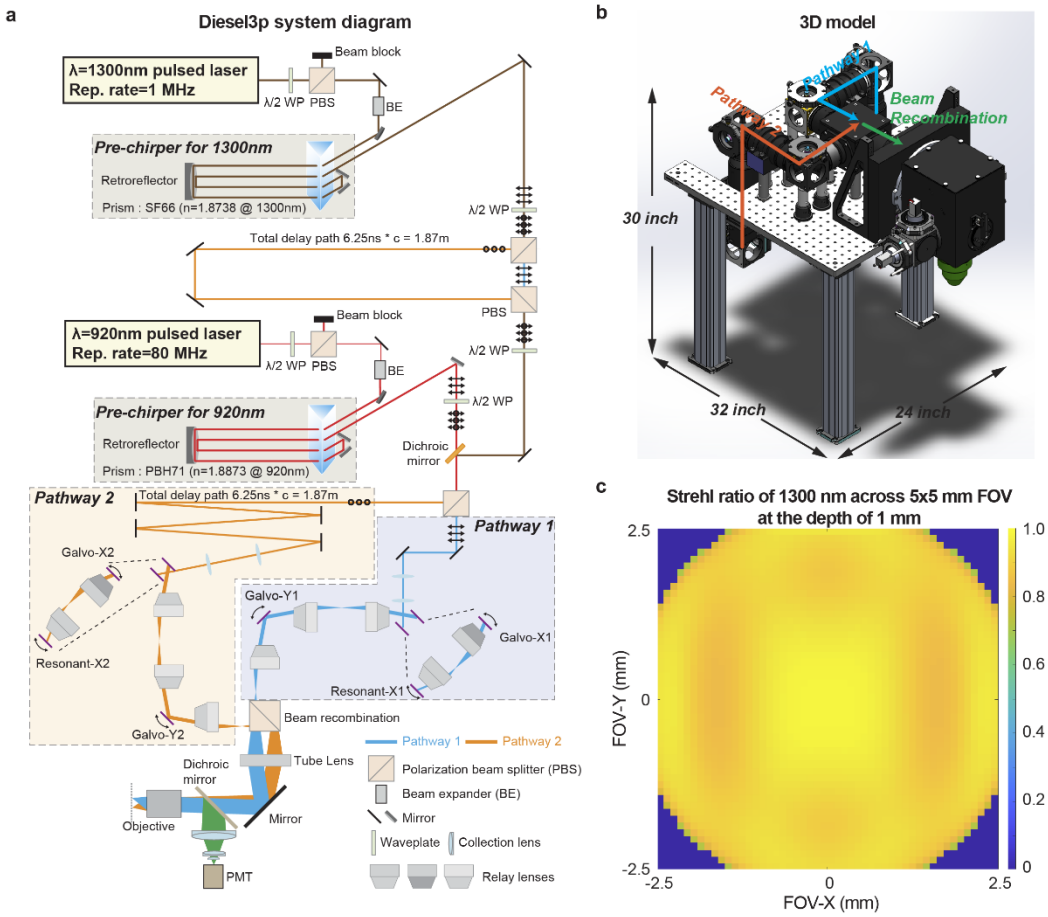
Support: NSF 1707287
NIH R01 MH111447

Title: A new mesoscope for deep tissue three-photon imaging in vivo

Authors: H. JAYAKUMAR[1], C.-H. YU[2], D. FARINELLA[1], E. TILSETH[1], S. L. SMITH[2], *P. KARA[1];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Electrical & Computer Engin., UC Santa Barbara, Santa Barbara, CA

Abstract: Different layers of the mammalian neocortex have very different functions and thus a complete functional architecture of the neocortex necessarily requires a description of functional maps and receptive field properties across all cortical laminae. Here we present a fully customized mesoscope with a field of view 5 × 5 mm, a Dual Independent Enhanced Scan Engines for Large Field-of-view 3-Photon imaging (Diesel3p) designed for large volume imaging of neurons and other structures across all layers of the neocortex. Based on our Diesel2p system developed for 2-photon microscopy (Yu et al. 2020 bioRxiv), the Diesel3p can be used in a variety of modalities. The two independent scan engines allow simultaneous two-region imaging with acquisition parameters independently configured for each region (see attached figure, panels a-b). Each arm can accommodate 920nm and 1300nm, so dual-region 2-photon or 3-photon imaging is feasible. The system is thus not only ideal for imaging GCaMP and various fluorescent dyes, but also may be used for third harmonic generation (THG) imaging. Our mesoscope has custom-designed relay lenses, scan lenses, a tube lens and an objective that provides a large field of view with diffraction-limited excitation to imaging depths > 1 mm. Based on the optical design model, the system has a Strehl ratio > 0.8 for 1300 nm light across a region of 5 × 5 mm, at a depth of 1 mm (see attached figure, panel c). Simultaneous imaging is implemented by temporal multiplexing of the excitation laser pulses from the two independent scan engines. We use a Vidrio Technologies DAQ and ScanImage software for demultiplexing the signals across the two scan engines. This mesoscope will help identify laminar-specific and regional-specific neural computations along with hemodynamic responses in a variety of model

systems. Thus, in addition to advances in neural circuit mapping and functional architecture, this system may help determine the neural and vascular underpinnings of signals that contribute to functional magnetic resonance imaging (fMRI).



(a) System layout, (b) 3D CAD model of the Diesel3p system, (c) Strehl ratio of the system for 3-photon excitation.

Disclosures: H. Jayakumar: None. **C. Yu:** None. **D. Farinella:** None. **E. Tilseth:** None. **S.L. Smith:** None. **P. Kara:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.03

Topic: D.07. Vision

Support: NIH Grant MH111447
NSF Grant 1707287

Title: Advancing laser standards for three-photon microscopy

Authors: *D. FARINELLA, A. ROY, C. J. LIU, P. KARA;
Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Three-photon excitation microscopy has double-to-triple the penetration depth in biological tissue over two-photon imaging and thus has the potential to revolutionize the visualization of biological processes in vivo. However, unlike the plug-and-play operation and performance of lasers used in two-photon imaging, lasers used in three-photon microscopy present new technological challenges that require a closer look at the fidelity of laser pulses. We built a second harmonic generation frequency-resolved optical gating (SHG-FROG) device (Fig. 1a), and developed and implemented a new technique called deep-memory diode imaging (DMDI) (Fig. 1d) to more fully characterize the pulse shape and pulse-to-pulse intensity stability of our commercial laser source, respectively. SHG-FROG revealed a large pulse distortion called third-order dispersion (TOD), which poses significant challenges to three-photon imaging by preventing effective compression of laser pulses and thereby decreasing the efficiency of nonlinear excitation (Fig. 1b,c). Additionally, DMDI demonstrated its ability to detect pulse-to-pulse intensity fluctuations in the laser source itself that were shown to be the cause of brightness artifacts in microscope images and were not captured using conventional diagnostics and statistics (Fig. 1e,f). If three-photon imaging is to become mainstream, these tools may be used by laser manufacturers to improve system design and by end-users to validate the performance of their current and future three-photon imaging systems.

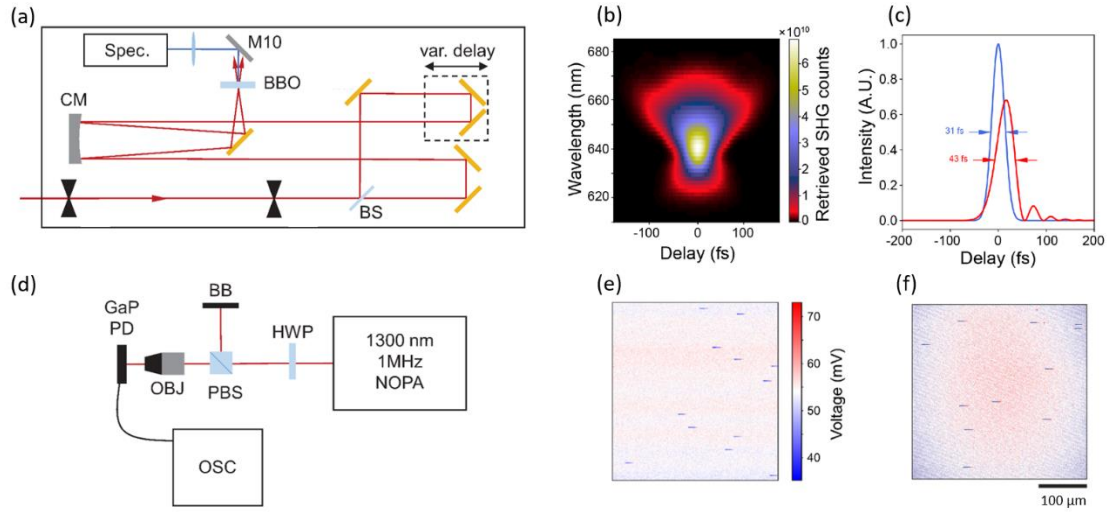


Fig. 1 (a) SHG-FROG schematic. (b) Measured FROG trace. (c) Simulated laser pulse with TOD (red) and no TOD (blue). (d) DMDI schematic. (e) Deep memory diode image. (f) Microscope image.

Disclosures: D. Farinella: None. **A. Roy:** None. **C.J. Liu:** None. **P. Kara:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.04

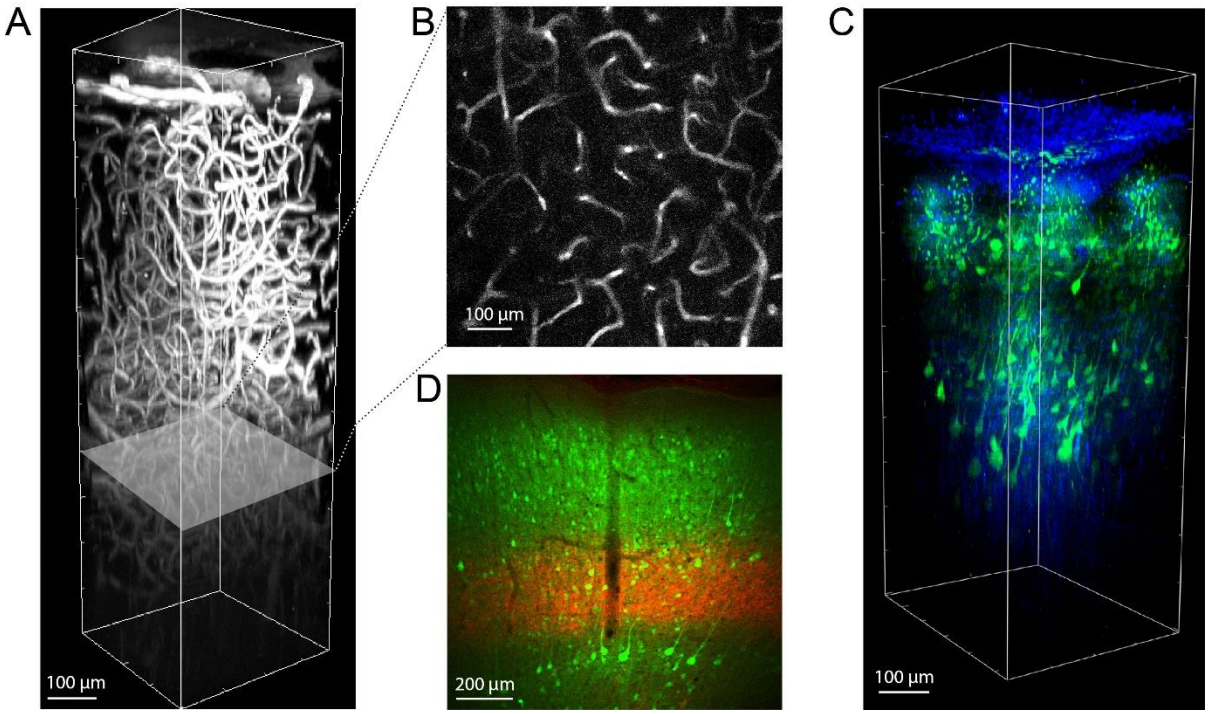
Topic: D.07. Vision

Support: NIH R01 MH111447
NSF 1707287

Title: Strategies to determine the functional micro-architecture in non-rodent cortical layer 4

Authors: *A. LEIKVOLL, A. ROY, C. J. LIU, P. KARA;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: In V1 of non-rodents (cats, ferrets and monkeys) receptive field properties such as orientation selectivity and binocular disparity emerge *de novo* in cortical layer 4. To date, these receptive field properties have only been determined with single-unit electrophysiological recordings. Thus, the functional micro-architecture of orientation, disparity and other maps in layer 4 remains unknown. Here we take advantage of the well-known neural and vascular functional architecture in layer 2/3 of cat V1 to determine if an equivalent architecture is present in layer 4. Moreover, because hemodynamic signals are often used a proxy for neural activity, we also determine the response selectivity of blood flow in individual layer 4 vessels. Critically, there are two major technical hurdles that need to be overcome in order to examine the functional architecture in layer 4. First, the laminar thickness of non-rodent V1 is at least twice that of mouse V1. In cat V1, we found that layer 4 is 650-1150 μm below the pial surface. This depth is inaccessible for two-photon GCaMP and iGluSnFr imaging. Also, typical AAVs with synapsin promoters do not lead to robust expression in layer 4 neurons (see Liu et al. 2020 Scientific Reports). We therefore developed three-photon microscopy for imaging in layer 4 and tested a variety of AAVs that optimally express in layer 4. As shown in the attached figure, we can successfully image in cat layer 4 with high signal-to-noise in both blood vessels (panels A-B) and neurons (panel C, green GCaMP6 neurons and blue THG). Our histology shows good expression in layer 4 (panel D, green GCaMP6 neurons and red VGLUT2 labeling LGN terminals). We will present data to compare neural and vascular responses across layers 2/3 and 4. The changes in selectivity across cortical layers for individual blood vessels appear to have a clear underpinning in neural circuitry. We expect that our three-photon imaging approach will facilitate the development of neural-to-vascular coupling models that map features of neural activity into predictions of activity observed for individual fMRI voxels.



Disclosures: A. Leikvoll: None. **A. Roy:** None. **C.J. Liu:** None. **P. Kara:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.05

Topic: D.07. Vision

Title: Visually-evoked responses from individual blood vessels in the white matter below V1

Authors: *A. BHALERAO, C. J. LIU, P. KARA;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Neural activity causes localized changes in blood flow. This neurovascular coupling is the basis for blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD fMRI). Historically, fMRI studies on the neocortex have focused on the gray matter and undervalued BOLD signals in the white matter, whether that be ignoring them or using them for noise correction (Grajauskas et al., 2019 Front. Neurosci.). We have obtained optical imaging data from individual blood vessels which suggest that the past approach may need to be revised. We first used two-photon imaging to examine whether there were visually-evoked blood flow

responses (measured as red blood cell velocity) in the white matter below the primary visual cortex in mice. We also collected visually-evoked responses from pial and intra-cortical blood vessels. All vessels were imaged using Alexa 680 Dextran. We presented drifting square-wave grating stimuli in either a 3 × 3 or 4 × 4 grid of positions on the visual display monitor. Stimuli were presented for 5 s with 20 s of blank in between presentations and repeated 4-6 times. In a subset of experiments, we also obtained three-photon third harmonic generation (THG, 1300 nm) signals to verify that the vessels were indeed in the white matter based on the strong intrinsic THG signal of axons (Liu et al., 2020 Sci. Rep.). We recorded blood velocity responses from white matter vessels at depths ranging from 729-920 μm below the pial surface. The diameter of these vessels ranged from 4-35 μm. We show for the first time that many white matter vessels demonstrate significant increases in blood flow in response to visual stimulation. Furthermore, several of these white matter vessels had clear retinotopically-selective responses such that a spatial receptive field could be mapped. The parent collaterals of the imaged white matter vessels may be principal cortical veins that dive in through the visual cortex gray matter or white matter vessels that bypass the imaged cortical region. To determine such anatomical categories of white matter vessels, we collected anatomical Z-stacks of the pial to white matter vessel volumes and we are currently performing quantitative reconstructions on these volumes. The functional significance of our new findings of visually-evoked and retinotopic responses in white matter vessels remains to be determined. However, they do suggest that white matter BOLD responses should not be treated as a proxy for physiological or machine noise. Assuming sufficient voxel resolution/magnetic field strength and an appropriate hemodynamic response function, the response properties of white matter voxels need to be thoroughly investigated in fMRI.

Disclosures: A. Bhalerao: None. **C.J. Liu:** None. **P. Kara:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.06

Topic: D.07. Vision

Support: NIH Grant R01EY030200
NIH Grant R00MH099654

Title: Sex and estrous cycle have minimal effects on physiology or experience-dependent plasticity in mouse primary visual cortex

Authors: *C. JENSEN[1], R. W. SCHECTER[2], J. GAVORNIK[1];
[1]Boston Univ., Boston, MA; [2]Sage Therapeut., Cambridge, MA

Abstract: It's been said that men are from Mars and women are from Venus (John Gray, PhD), but does this translate to the neurobiology of male and female mice? Most experimental neuroscience has been conducted using male animals. Various neuromodulators, including the hormone estrogen, can fundamentally alter plasticity induction. A report (Jeong et al. 2011) showing that V1 produces, and is sensitive to, estrogen suggests that functional physiological processing and learning from visual information may be sexually dimorphic. It is not clear from existing literature the extent to which sex chromosomes and the estrous cycles introduces variability in female mice that is not found in males. The recent NIH directive to include sex as a factor in vertebrate research also raises practical issues about how to properly control for variations and apply existing knowledge to sexually heterogeneous populations. For these reasons, we performed a series of in vivo electrophysiological experiments to characterize baseline visual function and several forms of experience-dependent plasticity in the primary visual cortex of male and female mice. We find a high degree of estrous cycle variability that is difficult to effectively control for in multi-day yoked experimental designs. However, while the literature suggests that underlying plasticity mechanisms are likely influenced by estrogen levels, we find no statistical difference in baseline function or functional experience-dependent plasticity relative to sex or estrous cycle in any of our assays. Though more investigation is necessary to explain why specific cellular-level sexual dimorphisms reported in previous studies are not obviously responsive to natural estrous cycles in vivo, we conclude that the cumulative endpoint of these plasticity processes is minimally influenced by gonadal hormones. Our findings suggest that female mice can be included alongside males in experiments focused on early visual processing or adult visual plasticity with no additional controls required.

Disclosures: C. Jensen: None. **R.W. Schecter:** None. **J. Gavornik:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.07

Topic: D.07. Vision

Support: NIH Grant R01EY030200

Title: M2 receptors are required for spatiotemporal sequence learning in mouse primary visual cortex

Authors: S. SARKAR, G. FERRON, C. MARTINEZ-REYEZ, C. JENSEN, *J. GAVORNIK; Boston Univ., Boston, MA

Abstract: Acetylcholine is a neurotransmitter that plays a variety of roles in both the central and peripheral nervous systems. In the CNS, ACh can act as a neuromodulator and and is known to

be involved in both visual perception and visuocortical plasticity (Kang et al., 2015). Our lab has previously shown that spatiotemporal sequence learning, defined here as the ability to encode and predict the spatiotemporal content of visual information, requires muscarinic signaling in the mouse primary visual cortex (V1, Gavornik and Bear, 2014). Muscarinic signaling is a complex process involving the combined activities of five different G-protein coupled receptors, M1-M5, all of which are expressed in the brain but differ from each other functionally and in anatomical localization (Groleau et al., 2015). Here we present electrophysiological evidence that M2, but not M1, receptors are critical for spatiotemporal sequence learning in mouse V1. We show that M2 is highly expressed in the neuropil in V1, especially in thalamorecipient layer IV, also is co-localized to the soma of a subset of somatostatin expressing neurons in both deep and superficial layers. We also show that expression of M2 receptors is relatively higher in the monocular region of V1 than than it is in the binocular region, but that paradoxically the amount of experience-dependent sequence potentiation that occurs in the monocular cortex is less than that seen in the binocular cortex. This work demonstrates that monocular circuits encode the same types of information as do binocular circuits and raises interesting questions about the functional role of M2-type receptors in processing temporal information with potential relevance to understanding how degradation of cholinergic neurons impacts cognitive function.

Disclosures: S. Sarkar: None. **G. Ferron:** None. **C. Martinez-Reyez:** None. **C. Jensen:** None. **J. Gavornik:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.08

Topic: D.07. Vision

Support: NIH Grant R01EY030200
NIH Grant R00MH099654

Title: Statistics of Temporally Ordered Stimuli Modify Activity in the Primary Visual Cortex

Authors: *S. KNUDSTRUP[1], J. P. GAVORNIK[2];
[2]Biol., [1]Boston Univ., Boston, MA

Abstract: Learning neural representations of regularities in the environment is critical for behavior (Ma and Jazayeri 2014). Temporal regularities are particularly important as they can infer causation and allow predictions about future events, and predictive coding may be a mechanism used by cortical circuits to detect and respond when the external environment violates learned expectations (Keller and Mrsic-Flogel 2018). The work from multiple labs suggests that V1 circuits are capable of building local predictive models of visual experience,

though the degree to which statistical predictability in the environment shapes these models is unclear (Bear, Buonomano, Carandini, Fiser, Gavornik, Hawkins, Keller, Shouval). To address this issue, we investigated how neurons in the mouse primary visual cortex (V1) respond during exposure to sequences of visual stimuli with a non-deterministic order governed by a set of transition probabilities. Using two-photon microscopy, we analyzed visually evoked neural activity at the population level as a function of transition probability, comparing responses elicited by higher and lower probability transitions. Here we will report how neural firing is modified and scales with transition probability and the time course of these modifications as the mouse accumulates exposure to various image sequences.

Disclosures: S. Knudstrup: None. **J.P. Gavornik:** None.

Poster

P473. Methods for Determining the Structure and Function of Primary Visual Cortex

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P473.09

Topic: D.07. Vision

Support: NIH Grant 5R01 EY030200
NIH Grant 4R00 MH099654

Title: Unsupervised Predictive Learning in Mouse Primary Visual Cortex: Experimental Data and Statistical Modeling

Authors: *B. PRICE, C. JENSEN, A. KHOUDARY, J. GAVORNIK;
Boston Univ., Boston, MA

Abstract: Predictive processing is a general term for various theories of cortical function, which describe the ability of the nervous system to predict future sensory inputs and the sensory consequences of action (Keller & Mrsic-Flogel, 2018). Such predictive processing is useful for the efficient coordination of movement (Körding & Wolpert, 2004; McNamee & Wolpert, 2019; Wolpert, Ghahramani, & Jordan, 1995), extrapolation of trajectories (Montague & Sejnowski, 1994; Palmer et al., 2015), and information compression (H. Barlow, 2001; Srinivasan, Laughlin, & Dubs, 1982), among others. In the present study, we investigate predictive processing in the context of sequence learning. Sequence learning is an unsupervised learning protocol in which repeated exposure to a visual stimulus induces a novel from of acetylcholine-dependent plasticity in V1 (Gavornik & Bear, 2014). In particular, for several days mice passively observe a sequence of rapidly-flashed sinusoidal gratings at about 7 Hz. Based on the principles of predictive processing and previous research within our lab, we hypothesized the visual system would learn a representation of the trained sequence that allows it to predict the order and timing of sequence elements. Under this framework, when an unexpected stimulus is presented or an expected

stimulus omitted, the mismatch between expectation and reality generates a so-called prediction error. Using sequence learning to induce expectations, we present spiking data from a cohort of about 50 adult mice suggesting that both positive and negative prediction errors are present in layer 4 of binocular V1. In addition, we use a statistical model (model-based targeted dimensionality reduction, Aoi & Pillow, 2018) to analyze how neural dynamics change with learning and the extent to which prediction errors are tuned to the precise spatiotemporal structure of the learned sequence.

Disclosures: B. Price: None. **C. Jensen:** None. **A. Khoudary:** None. **J. Gavornik:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.01

Topic: E.03. Basal Ganglia

Support: NIH Grant R01-DA045783

Title: Nucleus accumbens D1-positive neurons show escalating, reward-expectation-dependent spiking during motivated approach

Authors: *T. FAUST[1], A. MOHEBI[2], M. DUHNE[3], J. D. BERKE[1];
[2]Neurol., [1]UCSF, San Francisco, CA; [3]Neurociencia Cognitiva, Inst. De Fisiologia Celular UNAM, Mexico DF, Mexico

Abstract: A fundamental feature of basal ganglia circuitry is the distinction between two major subtypes of striatal projection neurons. About half express dopamine D1 receptors (D1+) while the other half express D2 receptors and adenosine A2a receptors (A2a+). Many studies manipulating these neuron subtypes have demonstrated distinct, opposite effects on behavior, yet their specific functional roles remain less than clear. Attempts to reveal these distinct functions by measuring their activity in behaving animals have described unexpectedly-similar patterns, with both subtypes increasing activity during action initiation.Using knock-in rat Cre driver lines (Pettibone *et al.* 2019), we recorded from optogenetically-identified D1+ cells (n=146, from 4 rats) and A2a+ cells (n=74, from 3 rats) in the nucleus accumbens during unrestrained performance of a reinforcement learning task (Hamid *et al.* 2016). In this task, rats dynamically adjust their action selection and willingness to work based on recent choice and reward history. In particular, when rats have recently received more rewards they are more motivated to begin a trial by approaching an illuminated port.As rats approached the trial initiation port, the D1+ neuron population progressively increased their firing, with greater initial firing rates corresponding to faster approach latencies. This ramping activity scaled with recent reward history, as quantified either by reward rate, or by state value in an actor-critic reinforcement

learning model. The timing of this ramp coincided with increased accumbens dopamine release, which also scales with reward history (Mohebi *et al.* 2019). By contrast, A2a+ neurons showed neither ramping during approach nor scaling with reward history. Neither D1+ nor A2+ neurons in accumbens showed substantial coding of left vs right choices, or the values of those actions.Our findings are consistent with accumbens D1+ neurons helping drive motivated approach behaviors, with this drive being promoted by dopamine release that scales with reward expectation.

Disclosures: T. Faust: None. **A. Mohebi:** None. **M. Duhne:** None. **J.D. Berke:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.02

Topic: E.03. Basal Ganglia

Support: NIH Grant R01-DA045783
NARSAD Young Investigator Award

Title: Coincident dopamine and acetylcholine ramps in the nucleus accumbens during motivated approach

Authors: *A. MOHEBI, J. D. BERKE;
Neurol., UCSF, San Francisco, CA

Abstract: Dopamine (DA) is a crucial regulator of both learning and motivation. Learning involves reward prediction errors, encoded by bursts of DA cell firing in the midbrain (including the ventral tegmental area, VTA). How exactly DA contributes to motivated behavior is less clear. Numerous studies have observed ramps in nucleus accumbens (NAc) DA release during motivated approach behaviors, that scale with reward expectation (value). Surprisingly, these value-related changes in the NAc DA release occur without any change in VTA DA cell spiking (Mohebi *et al.* Nature 2019). The specific mechanism(s) producing these DA ramps, and whether they are specific to NAc, is yet unknown. We used the DA biosensor dLight1.3b to measure DA dynamics across multiple subregions of the rat striatum: NAc (n=9 fiber placements), dorsomedial striatum (DMS, n=10), and dorsolateral striatum (DLS, n=10). We find that DA pulses to reward predictive cues covary with RPE in each subregion. However, ramps were only present in NAc, and not DLS or DMS - demonstrating a clear dissociation between ramps and RPE coding.In brain slices and anesthetized animals, striatal cholinergic interneurons (CINs) have been shown to locally regulate DA release. We therefore hypothesized that NAc DA ramps are driven by CINs, and recorded CIN activity using the calcium indicator GCaMP6f and fiber photometry (n=8 ChAT-Cre rats). We observed fast ramps in CIN activity, that mirrored the DA

ramps unaccounted for by VTA dopamine cell firing. Furthermore, we established that cholinergic modulation of DA release operates in freely-moving rats. We expressed ChR2 in NAc CINs and stimulated these neurons while monitoring dopamine DA through the same optic fiber using the red-shifted dopamine sensor RdLight. We observed a robust increase in dopamine that scaled with the duration and intensity of stimulation (n=4 rats). Finally, simultaneous monitoring of CIN GCaMP and DA using RdLight showed rapid, bidirectional interactions between DA release and CIN activity, with DA suppressing CINs and CINs enhancing DA release. These results support the hypothesis that motivation-related ramps in NAc release are locally controlled by CINs.

Disclosures: A. Mohebi: None. **J.D. Berke:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.03

Topic: E.03. Basal Ganglia

Support: NIH
UCSF

Title: Nucleus accumbens dopamine ramps reflect dynamic value estimates of possible future goals.

Authors: *T. KRAUSZ[1], A. COMRIE[1], L. M. FRANK[3], N. D. DAW[4], J. D. BERKE[2]; [1]Neurosci. Grad. Program, [2]Neurol., UCSF, San Francisco, CA; [3]Dept. of Physiol., UC San Francisco, San Francisco, CA; [4]Psychology, Princeton Univ., Princeton, NJ

Abstract: Dopamine (DA) in the nucleus accumbens (NAc) has been identified as a critical motivational signal. Our lab has found that DA correlates with estimates of future reward (value) as rats perform an operant reinforcement learning task. Consistent with a value signal, multiple groups have observed that NAc DA increases in a ramp as rats approach a distant goal. In many natural environments, however, there exist numerous navigational choices that lead to distinct rewarding locations. How does the NAc DA system estimate value in these contexts? We measured DA release dynamics in the NAc using the fluorescent biosensor dLight1.3b in Long-Evans rats (n=5) as they navigated for reward in a novel naturalistic foraging task, the Triangle Maze. This task leverages probabilistic reward delivery and movable barriers to vary the benefits and costs from discretized maze locations. Reinforcement learning algorithms allow us to estimate state values on a trial-by-trial basis. Rats adapted their navigational decisions to track changes in expected rewards and distance costs. State value was a significantly better predictor of DA dynamics during navigation than behavioral metrics such as velocity or acceleration. NAc

DA most strongly correlated with estimates of value for the chosen goal. However, early in a run, DA also correlated with values of the most rewarding alternative. Our results support the hypothesis that NAc DA is an evaluative signal, and they provide insight into how the brain assigns credit to states distant from reward.

Disclosures: T. Krausz: None. **A. Comrie:** None. **L.M. Frank:** None. **N.D. Daw:** None. **J.D. Berke:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.04

Topic: E.03. Basal Ganglia

Support: NIH
CHDI
UCSF

Title: Reward prediction error signals from slow pacemaker neurons in the globus pallidus

Authors: M. A. FARRIES[1], T. W. FAUST[2], A. MOHEBI[2], J. D. BERKE[2];
[1]Knoebel Inst. for Healthy Aging, Univ. of Denver, Denver, CO; [2]Neurol., UCSF, San Francisco, CA

Abstract: The globus pallidus pars externa (GPe) is a central node within basal ganglia circuits. GPe neurons fall into distinct subtypes based upon anatomical connectivity and expression of molecular markers, but identifying these subtypes in extracellular recordings in behaving animals has been challenging. We previously reported (Mallet *et al*. 2016) that "Arkypallidal" neurons (Arky; projecting solely to striatum) can be distinguished from "Prototypical" (Proto) cells by their more irregular firing and diminished spiking during slow-wave-sleep. Here, we recorded from many individual GPe neurons (n=1,367) in freely-behaving rats across the sleep-wake cycle, and during performance of Pavlovian and reinforcement learning (RL) tasks. We again found a clear distinction between Arky and Proto GP neuron clusters; using optogenetic tagging we confirmed that parvalbumin-positive neurons are near-exclusively found in the Proto cluster. Furthermore, we observed a novel, smaller population of cells (n = 96) with low firing rates (4.8 ± 1.8 Hz) and regular spiking (CV: 0.59 ± 0.15); we refer to these cells as "slow pacemakers." The firing patterns of these cells showed striking similarities to midbrain dopamine neurons recorded in the same tasks (Mohebi *et al*. 2019). In particular, they showed brief increases in firing to Pavlovian cues, in proportion to the cued reward probability, and brief increases in firing at reward delivery in inverse proportion to the cued reward probability. In the RL task, slow pacemakers showed a brief increase in firing at reward delivery that scaled inversely with

recent reward rate - i.e. it was stronger when rats had less expectation to reward - consistent with a positive reward prediction error. Slow pacemakers also responded to other cues in the RL task (trial initiation light, *Go!* trigger sound) in a very similar manner to midbrain dopamine neurons, and with similar timing.
Our results demonstrate a second source of reward prediction error signals within the basal ganglia, supplementing and potentially contributing to reward prediction error coding by midbrain dopamine cells.

Disclosures: M.A. Farries: None. **T.W. Faust:** None. **A. Mohebi:** None. **J.D. Berke:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.05

Topic: E.03. Basal Ganglia

Title: Behavior-related activity of optogenetically-identified ChAT+, PV+, and SST+ interneurons in the rat striatum.

Authors: *M. DUHNE[1], J. R. PETTIBONE[2], A. MOHEBI[1], T. W. FAUST[1], J. D. BERKE[3]; [1]Neurol., UCSF, San Francisco, CA; [2]Suomi Col. of Arts and Sciences, Finlandia Univ., Hancock, MI; [3]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: The striatum is an essential structure for adapting behavior to changing reward conditions. Local circuits within the striatum have a critical role in orchestrating striatal output. Activity in these circuits is heavily influenced by striatal interneurons, including cholinergic (ChAT+), parvalbumin-positive (PV+), and somatostatin-positive (SST+) cells. Dysfunction of these striatal interneurons is linked to a range of neurological disorders such as Parkinson's Disease, Huntington's Disease and Tourette Syndrome. Yet, exactly how local striatal circuits work remains poorly understood. *In vivo* recordings in behaving animals have found distinct neuronal subpopulations, such as "tonically-active neurons" presumed to be ChAT+ and fast-spiking cells presumed to be PV+. However, positive identification of striatal interneurons in behaving animals has proven extremely challenging, and the spiking activity of even presumed SST+ interneurons has not been reported at all. We have developed enhanced methods for optogenetic identification of extracellularly-recorded neurons in behaving rats. Using this approach we have begun to examine and compare the activity patterns of identified PV+, ChAT+ and SST+ interneurons in dorsal striatum, as rats perform Pavlovian and instrumental tasks. In awake rats (n=3 PV-Cre; n=3 ChAT-Cre; n=2 SST-Cre) all three cell types are consistently tonically-active, confirming that tonic activity alone is a poor guide to cell type. All three cell types also showed complex movement-dependent changes in firing rate, but only ChAT+ showed brief pauses with reward-related cues. These pauses were selectively observed in dorsal-

lateral, but not dorsal-medial striatum. Our results confirm that only a fraction of "tonically-active neurons" are ChAT+, demonstrating that positive identification will be very important for deciphering the operations of striatal microcircuits during behavior.

Disclosures: M. Duhne: None. **J.R. Pettibone:** None. **A. Mohebi:** None. **T.W. Faust:** None. **J.D. Berke:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.06

Topic: E.03. Basal Ganglia

Title: Midbrain inhibitory modulation of exploratory behavior and value-based action selection through dopamine neurons

Authors: *Z. ZHANG, N. K. GUT, J. MENA-SEGOVIA;
Behavioral and Neural Sci., Rutgers Univ., Newark, NJ

Abstract: The pedunculopontine nucleus (PPN) has traditionally been regarded as a midbrain motor structure. However, emerging evidence suggests that PPN influences behavior beyond locomotor control through its projections to areas of the basal ganglia involved in value processing, salience and novelty, thus supporting its broader involvement in the basal ganglia network. We previously reported a GABAergic PPN projection that innervates the substantia nigra pars compacta (SNc) and selectively affects goal-directed behavior, but not general motor programs. Dopamine has been shown, in animal models as well as computational studies, to affect the exploration-exploitation trade-off by mediating the basal ganglia output. To further explore this connectivity in the framework of dopamine-mediated exploration-exploitation, VGAT-Cre transgenic mice were optogenetically stimulated during a battery of behavioral tests. Mice were bilaterally injected with a Cre-dependent construct encoding channelrhodopsin-2 or a fluorescent reporter (controls) in the PPN, and bilateral optic fibers were implanted above the SNc. We trained the mice in a runway task, where animals learned to run through a straight corridor to get a food reward at the end; animals were food restricted and over-trained. To perform the task efficiently, corrective measures were used to minimize exploratory behaviors such as stopping, sniffing and rearing. Thus, successfully trained mice walked between the entry port and the food reward area without interruption. During the test, mice were challenged with optogenetic stimulation at the midpoint of the corridor or while they were eating the reward once they reached the end of the corridor. Consistent with our previous results, stimulation during reward consumption did not affect exploitative behavior. However, stimulation at the midpoint of the corridor resulted in a significant reduction of speed, where animals exhibited markedly impaired locomotion, but still managed to reach the food reward. In contrast, stimulation in the

open field, where there was no association with reward, led to full locomotor arrest. Next, mice will be tested in a two-choice foraging task to further explore the role of PPN GABAergic neurons in the modulation of exploratory/exploitative behavior through their influence over dopamine neurons. Our results support a role for PPN GABAergic neurons in goal-directed behavior through the modulation of dopamine neurons.

Disclosures: Z. Zhang: None. **N.K. Gut:** None. **J. Mena-Segovia:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.07

Topic: E.03. Basal Ganglia

Support: FFOR Grant

Title: The dopaminergic encoding of task completion in dorsal striatum

Authors: *J. GALANAUGH[1], E. DIAZ-HERNANDEZ[1], A. SANCHEZ-FUENTES[3], F. TECUAPETLA[3], M. V. FUCCILLO[2];
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Abstract: While the dorsal striatum has been implicated in action selection and initiation, less is known about how this region contributes to the completion of motor tasks. Perturbations to the neural encoding of task completion may contribute to the manifestation of compulsive and impulsive behaviors seen in multiple neuropsychiatric disorders. Dopaminergic signaling within the basal ganglia has been implicated broadly in motor planning, learning, and action execution. Here we test the potential contributions of this dopaminergic signaling to the encoding of task completion in lateral and medial compartments of dorsal striatum (DLS & DMS). We sought to investigate these signals in these regions with an adapted completion sequence task in mice (n=20). In this task, mice are presented with 2 levers on different walls of an operant box and are expected to complete a sequence (4 presses) on the first lever (sequence lever) and report completion of this sequence on the second lever (report lever) after which a reward is delivered. We record DA activity in DLS and DMS during behavior via the GRAB-DA optical dopamine sensor. Errors in this completion task took the form of incomplete sequences (<4 presses) or extended sequences (>5 presses) and served as phenotypes of impulsivity and compulsivity respectively. By the last day of training, the animals achieved a high level of performance, accurately self-reporting completion of a normal sequence (4 presses on the sequence lever, 1 press on the report lever) for 70% of trials. In the remaining trials, mice performed extended and incomplete sequences 11% and 19% of the time respectively. Distinct patterns of DA activity

were associated with each lever in normal sequence trials, and preliminary data suggests this activity is uniquely modulated in impulsive and compulsive trial types. This work will broaden our understanding of striatal dopamine signals in signaling the completion of motor sequences.

Disclosures: J. Galanaugh: None. **E. Diaz-Hernandez:** None. **A. Sanchez-Fuentes:** None. **F. Tecuapetla:** None. **M.V. Fuccillo:** None.

Poster

P523. Systems Behavior III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P523.08

Topic: E.03. Basal Ganglia

Support: BRF Seed Grant
NIH NINDS K08-NS072183
NVidia GPU grant

Title: Dopamine neuron stimulation induces progressive context-dependent abnormal involuntary movements in healthy rats

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Abstract: Levodopa alleviates many Parkinson Disease (PD) symptoms, particularly bradykinesia and rigidity. With continued levodopa treatment, many patients develop levodopa-induced dyskinesias (LIDs) - abnormal involuntary movements (AIMs) characterized primarily by chorea. Clinically, LIDs depend on progression of striatal denervation and sensitization to repeated levodopa doses. However, the degree of dopamine denervation is correlated with the magnitude of levodopa-induced changes in striatal dopamine, raising the possibility that elevated striatal dopamine can induce AIMs independently of nigrostriatal degeneration. We optogenetically stimulated SNc dopamine neurons in healthy, non-Parkinsonian rats as they performed a skilled reaching task. Repeated dopamine neuron stimulation was sufficient to induce progressive AIMs in the absence of nigrostriatal denervation. Additionally, AIMs severity was modified by behavioral context. AIMs were milder in rats engaged in skilled reaching during stimulation, and more severe if stimulation occurred between reaches. These data indicate that nigrostriatal denervation is not necessary for fluctuating striatal dopamine levels to induce AIMs, and that behavioral context plays a role in dyskinesia induction and expression.

Disclosures: J. Hunter: None. **A. Bova:** None. **D. Leventhal:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.01

Topic: F.02. Behavioral Neuroendocrinology

Support: Stanford UAR Small Grant
Stanford Biology Summer Undergraduate Research Program Grant
Rita Allen Foundation

Title: Neural regulation of juvenile aggression in a social tadpole

Authors: *J. MCKINNEY, S. LUDINGTON, J. BUTLER, L. A. O'CONNELL;
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Abstract: Aggression in juveniles is an evolutionarily adaptive behavior documented in many species, but the neural mechanisms behind these displays are poorly understood. In the Mimetic poison frog (*Ranitomeya imitator*), tadpoles live in small pools with limited resources and have evolved offensive aggressive tendencies towards conspecific tadpoles in defense of these resources. Tadpoles will attack, kill, and cannibalize other tadpoles as a primary resource defense mechanism. We examined the neural basis of neonate aggression in these tadpoles by comparing individuals that were placed in aggressive encounters and individuals placed in an environment with a non-threatening stimulus. We first did a longitudinal study to determine that the tadpoles were most aggressive when they were around five or six weeks old. We then compared patterns of generalized neural activity using immunohistochemical detection of phosphorylated ribosomes and a candidate neuropeptide, arginine vasotocin, which has been implicated in aggression in other vertebrates. Vasotocin cells in the preoptic area of the hypothalamus were not more active in aggressive tadpoles. We then examined neural activation across several brain regions suspected to be involved in aggression: the amygdala, ventral hypothalamus, preoptic area of the hypothalamus, and medial pallium. We found that the ventral hypothalamus had the most neural activation in aggressive tadpoles compared to controls and the other brain regions. To determine what cell types influence aggression, we used phosphoTRAP to moleculary profile active neurons in aggressive tadpoles. This approach identified proopiomelanocortin (POMC), a precursor polypeptide with multiple post-translational subunits, as enriched in active neurons in the diencephalon during aggression. Forkhead box protein 2 (FOXP2), a transcription factor involved in communication, was depleted in the telencephalon of aggressive tadpoles. Current work is focusing on doing pharmacological manipulations and neural tracing experiments to determine individual neuropeptide influence on aggressive behavior. Overall, this project is advancing our understanding of how aggression is regulated in the juvenile brain.

Disclosures: J. McKinney: None. **S. Ludington:** None. **J. Butler:** None. **L.A. O'Connell:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Grant IOS1656734
Texas Tech University Graduate School

Title: The effects of fluoxetine on approach and avoidance behavior by Xenopus laevis in a predator-based anxiety test

Authors: N. M. MENON, *J. A. CARR;
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Abstract: There are two neuronal pathways in mammals that detect and respond to visual threats: an LGN-striate cortex pathway and a subcortical pathway through the superior colliculus. Both pathways trigger defensive responses in the central nucleus of the amygdala through a thalamic relay. Here we use a novel ecologically relevant tradeoff test to investigate subcortical aspects of anxiety in anurans, which rely entirely on a subcortical visual processing pathway for responding to visual threats. We hypothesized that administration of fluoxetine (10 mg/kg administered alternate days for 30 d) may reverse anxiety-like behavior, improve feeding, and decrease abundance of anxiety-related transcripts in the African clawed frog *Xenopus laevis*. We divided adult male *X. laevis* into three groups: untreated, 0.6% saline, and fluoxetine injected i.p. (n = 9-10/group). After 30 d we placed individual frogs into the behavioral arena and co-presented a repeating looming threat and food, mimicking an ecological tradeoff where animals can forage at the risk of predation. We analyzed behavior during three test periods: a 10 min pre-test without either stimulus (A); a 30 min test-period with both the visual predator stimulus and food (B); and a 30 min post-test period with food only (C). After behavioral tests we euthanized frogs, flash froze optic tecta for RNA extraction, cDNA transcription and qRTPCR. Fluoxetine administration did not affect food intake relative to untreated and saline controls. Fluoxetine-treated frogs took significantly longer to start swimming in the tank during time period A compared to the other groups. Fluoxetine-treated frogs spent significantly less time swimming and more time in 'safe' side of the tank away from the predator in time period C compared to the untreated animals. We observed a significant increase in the abundance of transcripts for the serotonin 1A receptor and CRF in the vehicle-treated group compared to other groups. In summary, fluoxetine administration in *X. laevis* seems to enhance predator avoidance and promote sedentary behavior and did not improve exploratory in the recovery phase after exposure to a visual predator stimulus.

Disclosures: N.M. Menon: None. **J.A. Carr:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.03

Topic: F.02. Behavioral Neuroendocrinology

Support: DP2MH126375
R01MH126035
Alfred P. Sloan Fellowship

Title: Social rank predicts neural and behavioral responses to social stimuli

Authors: *D. R. BLACKMAN[1], A. R. MINERVA[1], O. C. TIMMERMANS[1], A. P. FINK[2], C. E. SCHOONOVER[2], A. L. FALKNER[1];
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Abstract: Social dominance hierarchies create ranking systems among conspecifics within the same social group. In order to achieve and maintain stable hierarchies, animals must engage in flexible and context-specific rank-appropriate behaviors. For example, dominants may approach and engage with conspecifics that subordinates will avoid. In addition, these behavioral responses must be flexibly updated if individual rank or social context changes. Here, we explore the role of a hypothalamic circuit in flexibly updating rank state and associated behaviors. To do this, we develop a novel assay to quantify behavioral responses to social sensory information as a function of rank. We combine this with quantitative behavioral characterization of variables related to aversion, engagement, and arousal. We find that in both sexes, social dominance hierarchies are linear, transitive, and stable, and behavioral responses to socially salient stimuli vary as a function of rank. Specifically, dominant animals show higher levels of engagement with these stimuli relative to subordinates; conversely, subordinates show higher levels of aversion relative to dominants. Further, we observe flexibility in these responses. Changes in social rank are correlated with corresponding behavioral changes: animals who ascend in the hierarchy show increased engagement, while animals who descend show increased aversion. To assess the neural correlates of these responses, we explored the role of a subpopulation of the ventral premammillary nucleus (PMvDAT), a glutamatergic node in the social behavior network that has been implicated in encoding both social rank and sensory information. We find that suppression o fthis node using DREADD Gi creates a subordinate-like state, changing behavioral responses to social and non-social stimuli to resemble the responses of subordinate individuals. In addition, fiber photometry results show that PMvDAT activity levels are higher in dominant animals relative to subordinates in both sexes, suggesting that social rank may modulate the gain of representations of social sensory information in the PMv. Together, these data provide an

understanding about how behavioral changes in rank state change computations at the neural level.

Disclosures: D.R. Blackman: None. **A.R. Minerva:** None. **O.C. Timmermans:** None. **A.P. Fink:** None. **C.E. Schoonover:** None. **A.L. Falkner:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.04

Topic: F.02. Behavioral Neuroendocrinology

Support: NIDA 4R00DA045662-02
NIDA P30 DA048736
NARSAD Young Investigator Award 27082
NIDA T32 5T32NS099578-04
NIH F31 1F31MH125587-01

Title: Dissociable neural mechanisms of reactive and appetitive aggression

Authors: *N. L. GOODWIN[1], L. BLOOM[2], K. PITTS[2], V. TSAI[2], Y. ZHANG[2], S. A. GOLDEN[3];
[1]Grad. Program in Neurosci., [3]Dept. of Biol. Structure, [2]Univ. of Washington, Seattle, WA

Abstract: Background Maladaptive aggression characterizes - or is comorbid with - many neuropsychiatric illnesses, and can have devastating effects on individuals, their caretakers, and healthcare professionals. Human aggression is typically demarcated as exhibiting either reactive (defensive) or appetitive (rewarding) components. Despite a significant clinical awareness of the differences between these aggression presentations, preclinical characterization of their relative circuitry and associated neuronal mechanisms are absent. Using outbred CD1 male mice, we examined the dissociable behavioral and neural mechanisms of reactive and appetitive aggression phenotypes.
Methods To cleanly dissociate appetitive and reactive aggression, we used operant aggression self-administration paired with yoked non-contingent aggression procedures, respectively. Briefly, for appetitive aggression, we trained male CD1 or CD1 transgenic mice to self-administer a novel subordinate intruder over 7 days using a trial design. In the reactive yoked condition, we non-contingently administered intruders with the same frequency distribution as the self-administration mice. To examine the neuronal ensembles engaged by aggression, residents were euthanized following testing and used for targeted Fos activity mapping with a focus on the lateral septum and anterior cingulate cortex. In parallel experiments, we used CD1xVgat-Cre or CD1xVglut1-Cre mice to examine cell-type specific activity in the lateral

septum.
Results Preliminary Fos activity mapping identified divergent patterns of neuronal ensemble engagement within the lateral septum.
Conclusions Preliminary results indicate that reactive and appetitive aggression differentially engage brain regions classically attributed to control only reactive aggression. Ongoing experiments will examine the endogenous population dynamics and causal role of these cell types in driving these aggression phenotypes.

Disclosures: N.L. Goodwin: None. **L. Bloom:** None. **K. Pitts:** None. **V. Tsai:** None. **Y. Zhang:** None. **S.A. Golden:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.05

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Z01 ES100221

Title: Hippocampal area CA2 activity supports resilience following an acute social stress

Authors: *D. RADZICKI[1], K. E. MCCANN[2], G. M. ALEXANDER[1], S. M. DUDEK[1];
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Abstract: Neuronal activity in the hippocampus is critical for memory acquisition and retrieval and influences an animal's response to stress. Moreover, the molecularly distinct principal neurons of hippocampal area CA2 are required for social recognition memory and aggression in rodents. Given the high concentration of the mineralocorticoid stress hormone receptor in area CA2, this region is also positioned to play an important role in the greater hippocampal stress response. To further interrogate the effects of stress on CA2 dependent behaviors, we chemogenetically manipulated neuronal activity *in vivo* during an acute, socially derived stressor and tested whether memory for the defeat was influenced.
We used the acute social defeat (ASD) paradigm where male subject C57 mice undergo a five-minute bout of social defeat in the home cage of a large male CD1 aggressor. 24 hours after the defeat, we observed that defeated mice spent, on average, significantly less time investigating a novel CD1 mouse when compared to control mice that had not been exposed to the aggressor mice (50.3 +/-10.5 vs. 99.4 +/-5.1 s, n=22,18, p=0.0001). Furthermore, we found that this avoidant phenotype persisted for up to one month following a single defeat: mice exhibited either a susceptible/avoidant or a resilient/investigative behavioral phenotype when CA2 activity was left unperturbed. When CA2 activity was inhibited using cell-type specific expression of the

inhibitory Gi-DREADD receptor during the defeat, however, subject mice exhibited a significantly higher amount of social avoidance one day later when compared to defeated littermates not expressing the DREADDs (241.6 +/-12.7 vs. 188.6 +/-16.4 s, n=18,18, p=0.02). This increased avoidance was driven by a loss of the 'resilient' population of defeated mice. We also observed ASD-dependent changes in neuronal activity following social investigation, as measured by the immediate early gene (IEG) c*fos*, in direct downstream targets of CA2 (rostral CA1, lateral septum). Lastly, given CA2's known role in aggression, we asked whether inhibition of CA2 also modulated the behavior of subjects during the defeat itself. We observed a significant reduction in the amount of defensive submission during defeat when CA2 was inhibited (24.5 +/-5.1 vs. 44.1 +/-6.3 s, n=18,18, p=0.02).
Taken together these results indicate that CA2 neuronal activity supports resilience following an acute social stressor and that submissive defensive behavior during the defeat (vs. fleeing) is a predictor of future resilience to social stress.

Disclosures: D. Radzicki: None. **K.E. McCann:** None. **G.M. Alexander:** None. **S.M. Dudek:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.06

Topic: F.02. Behavioral Neuroendocrinology

Support: UNAM GI200121
CONACYT CB-283279
NIMH/NIH MH02386

Title: Kolliker-fuse nucleus in the hindbrain parabrachial complex sends long-range glutamatergic (vglut1/2) projections containing pacap, cgrp and neurotensin, to identified gabaergic neurons in the extended amygdala during pain processing: new vistas of calyx-of-held-like synapse in the telencephalon of rat under focused ion bean scanning electron microscopy (fibsem) and transmission electron microscopy (tem)

Authors: *L. ZHANG[1], V. S. HERNANDEZ[2], D. M. GIRALDO[3];
[1]Dept. of Physiol., [2]Dept. of Physiology, Fac. of Med., [3]Fac. of Med., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: Kolliker (1886) - Fuse (1913) described a nucleus located in the dorsolateral pons in human that today carries their names (KFn). In rodent, it is located on the latero-ventral side of the superior cerebellar peduncle (scp) and comprises one of the three parabrachial subdivisions. It sends signals to the lower medulla oblongata, the spinal cord, the extended amygdala, and the

lateral hypothalamus. The KFn is more developed in humans than in other animal species. Chronic stimulation of the KFn region has resulted in relief of intractable pain in patients and the effect is comparable to that of PAG/periventricular stimulation. Using duplex and multiplex ISH, we report here a distinct neuronal population in the KFn that co-expresses VGLUT1, VGLUT2, PACAP, CGRP and neurotensin (NT) RNA. Using in vivo juxtacellular labeling, we observed that some of these neurons send long-range monosynaptic projections to the central amygdala (capsular division, CeC) and to bed nucleus of stria terminalis, oval division (BNSTov), making contact with PKCdelta immunopositive neurons co-expressing VGAT, PAC1 and VPAC2 mRNA, which were seen to project to lateral preoptic region, detected by in vivo labeled single neuron reconstruction. Using focused ion beam electron microscopy (FIBSEM) and transmission electron microscopy (TEM), we demonstrate for the first time that the PACAP-glutamatergic pathway from hindbrain establishes Calyx of Held-like synapses in extended amygdala and Gray type I synapses from PACAP immunopositive axons to GABAergic neuron somata. Fos activation was evaluated after formalin injection in the paws of urethane anesthetized rats. Strong neural activation was observed in the PBN / KFn and its targets in BSToval and CeC. Our results suggest the PACAP signaling pathway in KFn>CEC/BNSTov>LPO may serve as a "high-fidelity (HiFi)" circuit interaction for critical defensive locomotion toward survival.

Disclosures: L. Zhang: None. **V.S. Hernandez:** None. **D.M. Giraldo:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.07

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R15 MH122946

Title: Dominance status alters stress-related neural activity in medial amygdala circuits

Authors: *M. A. COOPER, M. S. JENKINS, M. K. HOOKER, C. J. WHITTEN; Psychology, Univ. of Tennessee Knoxville, Knoxville, TN

Abstract: Dominance hierarchies are a central aspect of mammalian social organization. While social subordination is associated with increased stress sensitivity, social dominance is linked with stress resilience. The neuronal mechanisms by which social dominance improves stress coping are not well understood and sex differences are poorly delineated. We used a Syrian hamster model to investigate the neuronal mechanisms controlling sex- and status-dependent differences in neural activity within the medial amygdala. We created dominance relationships by pairing same-sex individuals in daily dyadic encounters over a 12-day period. We also injected the retrograde tracer cholera toxin B (CTB) into the bed nucleus of the stria terminalis

(BNST). Then, we used the immediate early gene c-Fos as a marker for neural activity in the dorsal and ventral portions of the posterior medial amygdala (MePD and MePV) following the formation of dominance relationships and after social defeat stress. We hypothesized that dominant animals would activate a MePV-BNST pathway during both dominance encounters and social defeat stress. We found that both male and female hamsters formed stable dominance relationships with dominants showing elevated aggression and only subordinates showing submissive behavior. In males, but not females, dominants showed greater c-Fos immunoreactivity in MePD neurons projecting to the BNST during dominance encounters compared to subordinates. Similarly, male dominants showed greater c-Fos immunoreactivity in BNST-projecting MePD neurons during social defeat stress. These findings support the possibility that maintaining social dominance activates a MePD-BNST pathway in males, which allows for the sex-dependent activation of this neural circuit during acute social stress. Overall, this work provides insight into experience-dependent neural plasticity contributing to sex differences in stress resilience.

Disclosures: M.A. Cooper: None. **M.S. Jenkins:** None. **M.K. Hooker:** None. **C.J. Whitten:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.08

Topic: F.02. Behavioral Neuroendocrinology

Support: R15 MH122946

Title: The role of estrogen receptor alpha neurons in the medial amygdala in status-dependent differences in stress resilience in female hamsters

Authors: *M. K. HOOKER[1], C. J. WHITTEN[2], L. M. HENNON[2], M. A. COOPER[3];
[1]Univ. of Tennessee, Tennessee, TN; [3]Dept. of Psychology, [2]Univ. of Tennessee, Knoxville, TN

Abstract: While many people will encounter a traumatic experience during their lifetime, only 7-8 percent of those people go on to develop post-traumatic stress disorder (PTSD). Furthermore, the prevalence of PTSD in women is almost double compared to men. Unfortunately, much of the past neurobiological research regarding stress psychopathologies has focused on male subjects, leaving a translational gap when it comes to treatment options for women. Dominance status is known to influence stress responses with subordination being associated with increased stress sensitivity. In our laboratory we used a Syrian hamster (*Mesocricetus auratus*) model to better understand the mechanisms controlling status-dependent differences in stress resilience in females. This model allows us to create dyadic dominance relationships among female hamsters

over a 12-day period. Following establishment and maintenance of dominance relationships, females were subjected to social defeat followed by an approach/avoidance behavior test. To investigate the neuroendocrine mechanisms controlling status-dependent stress responses, we measured the activation of estrogen receptor alpha (ERα) immunopositive neurons within the posteroventral (MePV) and posterodorsal (MePD) medial amygdala. During the maintenance of dominance relationships, we found dominant females showed significantly increased attack rates compared to their subordinate counterparts while subordinates demonstrated higher flee rates compared to dominants. We found a main effect of defeat on social interaction time during an approach/avoidance behavior test; however, dominants did not differ from subordinates in their social investigation. Further, we found a main effect of defeat on social vigilance during approach/avoidance testing, although dominants did not differ from subordinates. Currently, we are using immunofluorescent staining to quantify cFos expression in ERα neurons within the MePD and MePV in dominant and subordinate female hamsters. By better understanding the neurobiological and environmental factors contributing to stress sensitivity in female animal models, we can better identify treatment options for stress-induced psychopathologies in women.

Disclosures: M.K. Hooker: None. **C.J. Whitten:** None. **L.M. Hennon:** None. **M.A. Cooper:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.09

Topic: F.02. Behavioral Neuroendocrinology

Support: R15 MH122946

Title: The role of androgen receptor-expressing neurons in the medial amygdala in the effects of dominance status on responses to social defeat stress

Authors: *C. J. WHITTEN[1], L. M. HENNON[1], R. M. RODRIGUEZ[1], J. E. KING[1], M. A. COOPER[2];
[1]Univ. of Tennessee Knoxville, Knoxville, TN; [2]Dept. of Psychology, Univ. of Tennessee, Knoxville, TN

Abstract: Social stress is a negative emotional experience that produces behavioral, physiological, and biochemical changes that leads to individual differences in fear and anxiety. Dominance status is an environmental factor that can alter the way individuals react to or cope with stressful events. The underlying neurobiology of how dominance status produces resistance to stress remains elusive. Androgen receptor (AR) expression has been shown to upregulate in stress-sensitive brain regions in dominant individuals compared to subordinate counterparts.

Using a Syrian hamster (*Mesocricetus auratus*) model we investigated whether dominant individuals display resistance to social defeat stress in a social anxiety test and whether they activate more AR-expressing medial amygdala postereodorsal (MePD) and postereoventral (MePV) neurons than their subordinate counterparts. We allowed male hamsters to form and maintain a dyadic dominance relationship for 12 days, exposed them to social defeat stress, and then tested their approach and avoidant behaviors in a novel environment. During the formation of dominance relationships, we found that dominant males showed a higher rate of attacks than subordinates and subordinates displayed a higher rate of flees compared to dominants. Following social defeat stress, we found a main effect on defeat such that those who were defeated spent significantly less time interacting with a novel conspecific, however, there was no effect of dominance status. Dominant and subordinate animals both showed greater social vigilance compared to animals not given the opportunity to from dominance relationships. We also found an effect on flank marking during a social anxiety test, such that non-defeated individuals flank marked more than animals exposed to social defeat. However, dominant and subordinate animals did not differ from each other in flank marking or social investigation. We are currently investigating status-dependent differences in activation of AR-expressing neurons in the MePD and MePV using immunofluorescent staining. This work can give insight to potential neural networks and biological mechanisms that govern status-dependent differences in stress responsivity.

Disclosures: C.J. Whitten: None. **L.M. Hennon:** None. **R.M. Rodriguez:** None. **J.E. King:** None. **M.A. Cooper:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.10

Topic: H.01. Attention

Support: NIMH K22-MH109558
NIH P20GM130461
NIH R34-DA050286

Title: Aggression associated with differential activity of neural regions implicated in retaliation

Authors: *A. MATHUR, R. ZHANG, J. BASHFORD-LARGO, J. ELOWSKY, M. DOBBERTIN, S. BAJAJ, K. BLAIR, J. BLAIR;
Boys Town Natl. Res. Hosp., Omaha, NE

Abstract: Background: While neuro-cognitive work examining aggression has examined patients with conditions at increased risk for aggression or individuals self-reporting past

aggression, little work has attempted to identify neuro-cognitive markers associated with observed/recorded aggression. The goal of the current study was to determine the extent to which aggression by youth in the first three months of residential care was associated with atypical responsiveness to retaliation.
Methods: This functional magnetic resonance imaging (fMRI) study involved 83 (56 male: mean age =16.18 [s.d. = 1.37]) adolescents in residential care performing a retaliation task during fMRI. Of the 83 adolescents participating in this study, 42 showed aggression within their first 3 months of residential care while 41 did not. During the retaliation task, participants were offered either a fair or unfair division of $20 pot (allocation phase) between the participant and imaginary partner, thereafter, the participants could either accept the offer or reject it and, by spending $1, $2, or $3 to punish the partner (retaliation phase).
Results: The post-hoc follow-up analysis (repeated measure ANOVAs) were conducted to identify association of aggression with atypical responding when retaliating to unfair offers for - (1) decision making regions representing expected value - vmPFC and posterior cingulate cortex (PCC) and (2) response control regions- particularly right IFG and bilateral anterior insula. The results of rmANOVA for decision making regions indicate significant differences in activity across the two groups of participants. The aggressive participants showed a significant less downregulation for retaliation phase for both vmPFC and PCC than the non-aggressive participants. Similarly, for response control regions, the aggressive participants showed less recruitment for retaliation phase as compared to non-aggressive participants.
Conclusion: On the basis of these results, we suggest that individuals with a greater propensity for aggression show reduced representation of the negative consequences of retaliation (reflected in the reduced down-regulation of vmPFC and PCC) and associated reduced recruitment of regions potentially involved in over-ruling these negative consequences in order to engage in retaliation (reflected in the reduced recruitment of right IFG and bilateral anterior insula).

Disclosures: A. Mathur: None. **R. Zhang:** None. **J. Bashford-Largo:** None. **J. Elowsky:** None. **M. Dobbertin:** None. **S. Bajaj:** None. **K. Blair:** None. **J. Blair:** None.

Poster

P609. Defensive Behavior and Aggression

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P609.11

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Grant #1748523
NSF Grant #1826871
NSF Grant #1832282

Title: Evaluating the ability of UNC-33L to rescue dauer formation in unc-33 mutants

Authors: *A. VACIO, J. CANTU, S. BECK, A. HOLGADO;
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Abstract: Aging in itself is a disease, and with aging comes susceptibility. Aging is an undeniable truth that introduces the body to additional diseases through macromolecular degeneration. Neurological diseases, such as Alzheimer's and Parkinson's, are due to the acceleration of neuronal degeneration that result in functional decline, deterioration of the body, and reduced standard of living. Although aging may be an undeniable truth for us, the nematode, C. elegans, can halt the aging process by molting into a stage termed "dauer." The *unc-33* mutant strain of the C. elegans, however, cannot enter into this stage, and the cause is unknown. The *unc-33* gene is responsible for axonal elongation, microtubule organization, and axon dendrite sorting all of which are essential for the establishment of an organism's nervous system. UNC-33 codes for three alternatively spliced isoforms UNC-33L, UNC-33M, and UNC-33S. Previous studies have shown that the isoform UNC-33L was able to rescue the *unc-33* mutant phenotype. To examine the ability of UNC-33L to rescue dauer formation, we examined *unc-33*(mn407) mutants expressing isoform UNC-33L and assessed them for dauer formation.The N2 wildtype, *unc-33*(mn407), and daf-7(e1372) were used as a control. To quantify and assess the ability of each strain to produce dauers, we used 1 percent SDS resistance assays. We conducted 3 separate trials for each strain and found that isoform UNC-33L fails to rescue the dauer formation defects characterizing unc-33(mn407) mutants. Quantification of 1 percent SDS resistance showed 73.14 percent of N2 wild type worms were dauers, while 96.68 percent of daf-7(e1372), 3.48 percent of *unc-33*(mn407) and 6.74 percent of *unc-33*(mn407) expressing isoform UNC-33L were dauers. Statistical analysis denoted that the percent of dauer formation was significant between the wild type and the strain containing the UNC-33L isoform. However, pairwise comparison of *unc-33*(mn407) mutants with *unc-33*(mn407) expressing isoform UNC-33L showed no statistically significant difference. Moving forward, as UNC-33L does not rescue the dauer defective phenotype of *unc-33* mutants, we will assess whether other UNC-33 isoforms regulate dauer formation. Together, these investigations look into the molecular machinery that slows down aging and minimizes susceptibility to age-related diseases; the mechanisms that are evolutionarily conserved from worms to humans.

Disclosures: A. Vacio: None. **J. Cantu:** None. **S. Beck:** None. **A. Holgado:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.01

Topic: F.07. Autonomic Regulation

Title: Investigating the vulvar blood flow response to short-term pudendal and tibial nerve stimulation in intact and spinalized rodents

Authors: *E. C. BOTTORFF, T. M. BRUNS;
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Abstract: Female sexual health is an important determinant in quality of life, contributing to both increased meaning in life and general well-being. Unfortunately, approximately 40% of women suffer from female sexual dysfunction (FSD). Neuromodulation is a potential treatment option for women with FSD. Some women receiving neuromodulation for bladder dysfunction have reported off-target benefits in sexual function. We recently studied neuromodulation in women with FSD but without bladder disorders and found that transcutaneous pudendal and tibial nerve stimulation improved FSD symptoms. We theorize that neuromodulation-driven improvements in FSD symptoms may occur via increases in blood flow to sexual organs.
We designed a set of anesthetized rat experiments (N = 10) to determine the impact of spinal cord transection on the peripheral circuitry modulating vulvar blood flow (VBF) responses to short-term stimulation, building upon prior studies on vaginal blood flow. For each 45-minute trial, we measured VBF with laser speckle imaging (arbitrary perfusion units; APU) for 5 minutes prior to stimulation, during 20 minutes of pudendal or tibial nerve stimulation, and for 20 minutes after stimulation ended. The trial was then repeated for the other nerve target. Next we performed a laminectomy and transected the spinal cord (T6 level). After a 15-minute or greater rest, we then repeated the two stimulation trials in the same order as before transection. The average VBF was 183 ± 44 APU prior to pudendal stimulation, which increased to 354 ± 110 APU ($p < 0.01$ versus prior) during pudendal stimulation and returned to 186 ± 53 APU post-stimulation. After spinal cord transection, mean VBF trends were similar (193 ± 61; 337 ± 131, $p < 0.01$ versus prior; 188 ± 65 APU, respectively). For tibial stimulation trials, VBF was 183 ± 50 APU pre-stimulation, 192 ± 48 APU during stimulation, and 181 ± 52 APU post-stimulation. After spinal cord transection, VBF remained steady at 192 ± 74, 211 ± 87, and 196 ± 65, prior to, during, and post-stimulation respectively.
These results suggest that single trials of short-term pudendal nerve stimulation have a direct effect on VBF, but short-term tibial nerve stimulation does not. The pudendal nerve stimulation response is likely due to direct activation of motor efferents that are not affected by the removal of supra-T6 spinal circuits. Further research is required to understand the impact of neuromodulation on changes in blood flow to the vulva and other genitals over a longer period time as the benefit of neuromodulation for FSD may improve with multiple sessions.

Disclosures: E.C. Bottorff: None. **T.M. Bruns:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.02

Topic: F.07. Autonomic Regulation

Support: Medtronic

Title: A behavioral animal model for non-continuous neuromodulation of bladder function

Authors: *M. ORTIZ-LOPEZ[1,2], Z. OUYANG[1,2], A. KOTKAR[1,2], M. WILLEN[1,2], R. LIU[1,2], J. OLSZEWSKI[1,2], A. RYBICKI[3,2], M. STEVEN[1,2], E. KENNEDY[1,2], T. M. BRUNS[1,2]; [1]Biomed. Engin., [2]Biointerfaces Inst., Univ. of Michigan, Ann Arbor, MI; [3]Dept. of Bioengineering, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Overactive bladder (OAB) is a medical condition that affects life quality, particularly for elderly individuals. Current sacral neuromoduation (SNM) therapy uses open-loop continuous stimulation to treat OAB symptoms. A system with a closed-loop or on-demand non-continuous strategy may increase therapeutic efficacy and efficiency. In this study we performed non-continuous nerve stimulation in an awake and behaving animal model, to expand upon prior studies performed with anesthetized animals. We implanted seven felines with microelectrodes in sacral-level dorsal root ganglia (DRG) towards real-time estimation of bladder pressure. In addition the animals were instrumented as follows; stimulation electrodes at one sacral nerve and a cuff electrode on a pudendal nerve for neuromodulation; catheters in the bladder for volume control and pressure monitoring; and fixed connections in a backpack mounted at the lower back. In the first three animals we established logistics for tethered neuromodulation and neural signal monitoring while animals were awake and moving around in an enclosure. In each of the following four test animals we performed seven separate test sessions across three-week periods. In each session the bladder was infused at least three times until the animal voided for each of three types of nerve stimulation: no neuromodulation, closed-loop neuromodulation initiated at DRG-estimated rising bladder pressures, and continuous neuromodulation. The choice of stimulation and sensing varied varied among experiments because of experimental issues such as failures of DRG microelectrodes and one test animal was removed from the study due to a health complication. In two test animals, closed-loop neuromodulation and non-continuous neuromodulation initiated at half-capacity and each had significantly larger bladder capacity than no-stimulation trials (37%, 32% respectively). These paradigms resulted in similar bladder capacity increases as continuous stimulation but used significantly less stimulation time (~50%). One animal was a non-responder to neuromodulation, with all nerve stimulation types yielding similar bladder capacities. The bladder voiding efficiency and maximum pressure change were not statistically different within the test animals across the different stimulation paradigms. This study demonstrates that neuromodulation can be delivered in a non-continuous paradigm that increases bladder capacity to a similar level as continuous neuromodulation in some awake and behaving felines. Further studies are needed to develop robust hardware and artifact-resistant algorithms.

Disclosures: M. Ortiz-Lopez: None. **Z. Ouyang:** None. **A. Kotkar:** None. **M. Willen:** None. **R. Liu:** None. **J. Olszewski:** None. **A. Rybicki:** None. **M. Steven:** None. **E. Kennedy:** None. **T.M. Bruns:** 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.; Medtronic.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.03

Topic: F.07. Autonomic Regulation

Support: Urology Care Foundation - Research Scholar Award Program # 666548
NIDDK grant R01 DK119183
NIDDK grant P30-DK079307

Title: Bladder Infection with Uropathogenic E. coli Increases the Excitability of Afferent Neurons

Authors: *N. MONTALBETTI[1], M. G. DALGHI[1], S. I. BASTACKY[1], D. R. CLAYTON[1], W. G. RUIZ[1], G. APODACA[1], M. D. CARATTINO[2];
[2]Med., [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Introduction: Urinary tract infections (UTIs) are among the most common bacterial infections affecting humans. Urinary urgency, frequency and pelvic pain are the main symptoms experienced by patients with UTI. The goal of this work was to define the contribution of afferent pathways to symptom generation in a mouse model of UTI induced by uropathogenic *E. coli* (UPEC). **Methods:** Female C57BL/6J mice were inoculated intravesically with UPEC strain UTI89. Immunofluorescence and histopathological analysis was conducted to characterize the infection and inflammatory process triggered by UTI89. Somatic sensitivity in the pelvic area was determined with von Frey filaments. Voiding was evaluated in conscious freely moving mice with a novel video analysis system. Electrical properties of bladder sensory neurons were evaluated with the patch-clamp technique. **Results:** Mice with UTI developed a robust inflammatory response with diffusive edema and leucocytic and lymphocytic infiltrate in the bladder mucosa. Mice with UTI exhibited a larger number of voids per hour and lower voided volume than controls inoculated with saline. Consistent with sensitization, bladder sensory neurons with both tetrodotoxin-resistant (TTX-R) and TTX-sensitive (TTX-S) action potentials harvested from mice inoculated with UTI89 exhibited aberrant firing in response to electrical stimulation. Spontaneous firing activity was observed in approximately 30% of bladder sensory neurons from mice with UTI. The incubation of naïve bladder sensory neurons with supernatants from UPEC cultures promoted the sensitization of neurons with both TTX-R and TTX-S neurons. Unexpectedly, lipopolysaccharide (LPS)-reduced supernatants only increased the excitability of bladder sensory neurons with TTX-R action potentials. **Conclusion** Our studies demonstrate that mice infected with UTI89 present with hallmarks of UTI including edema and

lymphocytic infiltration of the bladder wall, increased voiding frequency, and pelvic allodynia. The intravesical inoculation of UTI89 promoted the sensitization of bladder afferent sensory neurons with both TTX-R and TTX-S action potentials, supporting the notion that sensitized bladder afferents are the primary driver of symptoms in mice. Furthermore, our studies indicate that multiple virulence factors, besides LPS, likely contribute to the sensitization of bladder afferents.

Disclosures: N. Montalbetti: None. **M.G. Dalghi:** None. **S.I. Bastacky:** None. **D.R. Clayton:** None. **W.G. Ruiz:** None. **G. Apodaca:** None. **M.D. Carattino:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.04

Topic: F.07. Autonomic Regulation

Support: NIH SPARC OT2OD025297
SPARC Program

Title: Soft silicone-based neural interface to modulate bladder function

Authors: *R. KUMAR[1,2], C. GOPINATH[3,2], T. W. SIMPSON[3,2], D. M. WEIR[3,2], A. THIESSEN[4], D. MCDONNALL[4], R. A. GAUNT[3,2,1];
[1]Dept. of Bioengineering, [2]Rehab Neural Engin. Labs, [3]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [4]Ripple, Salt Lake City, UT

Abstract: Direct bladder wall stimulation has been attempted for decades to restore bladder function in people with spinal cord injury and other voiding dysfunctions. However, these efforts were limited by co-activation of the urethra, legs and other pelvic organs at stimulus intensities that evoked bladder contractions. Neural interfaces for the detrusor muscle itself face several challenges due to its structure and the volume changes it undergoes in normal function. We designed a stretchable silicone net that can be placed around the bladder body to anchor a soft electrode array that interfaces directly with the base of bladder to generate bladder contractions. We created implantable versions of the electrode nets and tested them in chronic experiments. We implanted 5 healthy cats (4 females, 1 male) and tested them with and without anesthesia for 2-3 months. A pressure catheter was implanted into the bladder to control volume and measure pressure. Bladder wall stimulation through various electrode configurations (monopolar, bipolar), temporal patterns (single electrode, sequential stimulation of multiple electrodes) and stimulus intensities were able to generate complete bladder emptying. In behaving cats, bladder wall stimulation at many different stimulus intensities elicited efficient voiding at physiological bladder pressures (med: 41, IQR: 27-60 cmH_2O). However, there was little relationship ($r<0.28$)

between stimulation amplitude and bladder pressure itself. Rather, stimulation amplitude had an effect on the time between stimulation onset and bladder contraction onset. At low stimulation amplitudes, there was a longer time between stimulation onset and voiding (med: 35s, IQR: 13.5-68.2s) than occurred at higher amplitudes, which evoked more rapid voiding (med: 23s, IQR: 10-45s). This contrasts with anesthetized tests, where stimulation at 3 ± 2 mA evoked increases in bladder pressure up to 35 cmH_2O. These results suggest that stimulation of the bladder wall recruits sensory pathways leading to an urge to void, but that can be suppressed by the animals. For most of the duration of the implants, stimulation evoked functional voiding with consistent electrical stimulation thresholds. However, after several months there was a failure of an implanted bond that limited the overall duration of the implants. Chronic experiments demonstrate that these electrode nets can be used as a neural interface to generate or initiate comfortable, complete bladder emptying in awake behaving cats. Future efforts include understanding the effects of evoked sensations on voiding and improving the integrity of the bonding between the flexible conductors and the lead wire.

Disclosures: R. Kumar: None. **C. Gopinath:** None. **T.W. Simpson:** None. **D.M. Weir:** None. **A. Thiessen:** None. **D. McDonnall:** None. **R.A. Gaunt:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.05

Topic: F.07. Autonomic Regulation

Support: NIH SPARC OT2OD028191

Title: Urethral recruitment during clinical pudendal neurostimulator implant surgery

Authors: *P.-J. CHEN[1], A. C. LAGUNAS[1], V. SORIANO[1], P. GUPTA[2], T. M. BRUNS[1]; [1]Biomed. Engin., [2]Urology, Univ. of Michigan, Ann Arbor, MI

Abstract: Patients with pelvic pain and/or urinary dysfunctions may receive a neurostimulator implant at the pudendal nerve, in an off-label use of sacral neuromodulation. During the implant surgery, surgeons typically monitor external anal sphincter (EAS) electromyography (EMG) responses to help determine the position of the four-electrode lead. The pudendal nerve also innervates the urethra. Monitoring urethral pressure responses during the implant surgery may also indicate lead positioning. Here, we use a multi-sensor catheter during the neurostimulator stage-1 implant at the pudendal nerve to map stimulation-driven pressure changes across the urethra. Each patient was situated for their implant surgery, with a multi-sensor catheter placed in the urethra to the bladder and EAS EMG needles inserted on either side of the anus. Once the neurostimulator lead was positioned near the nerve, we performed short 3 Hz stimulation trials of

different amplitudes (at and above the threshold level per electrode for driving EAS EMG, EASt), pulse widths (60, 210, 450 μs), and electrode combinations (E0 through E3). For each trial we are quantifying the average stimulation-driven pressure change (ΔP) on each urethra sensor. Our preliminary results are very diverse among our first five female patients: two patients had no urethral responses within our stimulation ranges, one patient had greater responses towards the proximal urethra, and two patients had greater responses towards the distal urethra. In the distal urethra responders, Patient 1 had pressure responses at EASt while responses in Patient 2 required at least twice EASt. In general, as we increased the stimulation amplitude, we observed larger distal and proximal urethra pressure increases. To our knowledge, this is the first multi-sensor recording of urethral pressure changes during clinical pudendal nerve stimulation. The difference in recruitment profiles among patients suggests that the relative electrode-to-nerve positions may vary across implant procedures. This mapping study will enhance our understanding of the spatial recruitment of the urethra by pudendal nerve stimulation in patients. Furthermore, this study may help improve the precision of neurostimulator lead placement and thus has the potential to benefit future clinical practice.

Disclosures: P. Chen: None. **A.C. Lagunas:** None. **V. Soriano:** None. **P. Gupta:** None. **T.M. Bruns:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.06

Topic: F.07. Autonomic Regulation

Support: NIH SPARC Program OT2OD023873

Title: Sparc - effects of sacral root stimulation on bladder and pelvic floor activity in awake, behaving cats

Authors: *D. BOURBEAU[1,2], S. MAJERUS[3], B. HANZLICEK[3], M. CAVENEY[4], A. RIETSCH[4], B. M. BALOG[4,6], **M. S. DAMASER**[5,7];
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Abstract: Introduction: Following spinal cord injury many individuals develop detrusor-sphincter-dyssynergia (DSD), which results in difficult bladder emptying due to discoordinated reflex urethral sphincter contractions. Sacral anterior root stimulation (SARS) has effectively

restored bladder emptying for thousands of individuals with SCI. For individuals with DSD, reflex sphincter contraction can impede bladder emptying. This problem has been addressed by the addition of a posterior rhizotomy of the sacral sensory nerves to eliminate the unwanted reflex sphincter activity as well as unwanted bladder reflexes for continence. Unfortunately, the rhizotomy also inhibits desirable reflexes, such as bowel and sexual reflexes, and removes any remaining sacral sensation. Therefore, there is a pressing need for alternatives to rhizotomy. In this study, we have tested the effects of sacral root stimulation to reduce urethral sphincter activity.
Methods: In N=6 cats, cuff electrodes were implanted bilaterally around sacral roots S1 and S2, and EMG electrodes were implanted bilaterally in the pelvic floor muscles. Pelvic floor EMG was used as a proxy for urethral sphincter activity. The UroMOCA, a wireless, catheter-free pressure sensor, was implanted in the bladder. During a 4-week chronic experiment, data were collected with the animal awake, behaving, and untethered. Tolerance limits were determined based on animal behavior. Bladder pressure and pelvic floor muscle EMG were recorded during normal activities, including bladder emptying events, and in response to sacral root stimulation with frequency, amplitude, and location randomized. Stimulation frequencies included 20 Hz, 500 Hz, and 10 kHz.
Results: Stimulation at 20 Hz activated pelvic floor muscles. Stimulation at 500 Hz resulted in a brief initial onset response in pelvic floor muscles, followed by a rapid, significant reduction in activity. 10 kHz stimulation resulted in an onset response and more rapid and significant reduction in muscle activity. Muscle fatigue was not observed. These results are consistent with the literature for animals under anesthesia. Some bladder responses were observed at the stimulation amplitudes tested, but the thresholds were significantly higher than for pelvic muscle.
Conclusion: Sacral root stimulation at 500 Hz or 10 kHz has potential to reduce unwanted urethral sphincter activity during bladder emptying. New wireless sensors allow detection of effect in conscious animals. These approaches should be further tested for possible clinical translation.

Disclosures: D. Bourbeau: None. **S. Majerus:** None. **B. Hanzlicek:** None. **M. Caveney:** None. **A. Rietsch:** None. **B.M. Balog:** None. **M.S. Damaser:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.07

Topic: F.07. Autonomic Regulation

Support: NIH SPARC OT2OD024908
Craig H. Neilsen Foundation – 476681

Title: High-resolution spinal cord stimulation produces micturition and continence reflexes in anesthetized and chronically-implanted awake behaving animals.

Authors: *C. GOPINATH[1,3], M. K. JANTZ[2,3], A. K. MC CUMBER[1,3], R. KUMAR[2,3], L. E. FISHER[1,3], C. CHIN[4], Z. HU[4], B. MCLAUGHLIN[5], R. A. GAUNT[1,3];
[1]Physical Med. and Rehabil., [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]Rehab and Neural Engin. Labs, Pittsburgh, PA; [4]Micro-leads Neuro, Somerville, MA; [5]Micro-Leads, Somerville, MA

Abstract: Epidural spinal cord stimulation (SCS) is a readily-translatable technology with recent demonstrations of its effectiveness in improving locomotion, alleviating high blood pressure and other dysfunction in people with spinal cord injury[1-3]. Our previous work showed that high-resolution SCS (HR-SCS) can target and recruit lower urinary tract nerves selectively[4], but these experiments were not designed to investigate the functional effects of stimulation. Here, we demonstrate that HR-SCS can evoke continence and micturition reflexes in both anesthetized and awake behaving cats at 90 days. We implanted HR-SCS electrodes in healthy chloralose-anesthetized animals for terminal procedures (n=6) and during sterile recovery surgeries to test performance in awake behaving animals (n=2). A bladder dome catheter was implanted for controlling volume and recording intravesical pressure. At constant volumes, low (3 Hz) and high frequency (33 Hz) stimulation was delivered (300-800 µA, 30-60 s, 200 µs pulsewidth) on all channels sequentially while monitoring for changes in bladder pressure. Responsive channels were then evaluated in filling and voiding cystometry trials. In implanted cats, trials were conducted bimonthly under dexmedetomidine anesthesia to identify channels producing changes in the bladder.Micturition and continence reflexes were evoked on responsive electrodes (53 bipolar channels; 6 cats) simply by altering the stimulus frequency. In terminal experiments, 33 Hz stimulation generated voiding pressures (27±5 cmH2O) with incomplete voiding in all animals. This voiding along with increase in pressure was abolished after a thoracic spinal transection in all animals. 3 Hz stimulation resulted in robust continence effects characterized by abolishment of non-voiding contractions and by decreases in bladder pressure in all animals (ΔP = -14±3 cmH2O) indicating relaxation of the detrusor muscle. This stimulation-evoked neural circuit was intact despite the transection of the thoracic cord. Similar frequency-dependent effects on the bladder were observed in chronic implanted free-behaving cats where 3 Hz stimulation produced a continence effect along with decreasing the desire to void measured by digging behavior. 33 Hz stimulation, which evoked large pressure increases in anesthetized animals, was largely ineffective in awake animals, possibly due to voluntary inhibition. These results indicate that HR-SCS could be an effective neuromodulation strategy, potentially even after spinal cord injury. Our future efforts are focused on investigating these in chronic spinalized animals as well as feasibility studies in clinical trials.

Disclosures: C. Gopinath: None. **M.K. Jantz:** None. **A.K. Mc Cumber:** None. **R. Kumar:** None. **L.E. Fisher:** None. **C. Chin:** A. Employment/Salary (full or part-time):; Micro-leads Neuro LLC. **Z. Hu:** A. Employment/Salary (full or part-time):; Micro-leads Neuro LLC. **B. McLaughlin:** A. Employment/Salary (full or part-time):; Micro-leads Neuro LLC. **R.A. Gaunt:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.08

Topic: F.07. Autonomic Regulation

Support: NIH SPARC Award OT2OD024898

Title: Restoration of bladder function with lumbosacral spinal cord epidural stimulation in persons with chronic spinal cord injury

Authors: *A. HERRITY[1], S. ASLAN[1], S. MESBAH[1], B. UGILIWENEZA[1], A. MOHAMED[1,2], C. HUBSCHER[1,3], S. HARKEMA[1,4];
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Abstract: One of the major challenges impacting overall health and quality of life following spinal cord injury (SCI) is the development of lower urinary tract dysfunction. Daily bladder management typically includes a combination of catheterization (either self-intermittent or suprapubic) and pharmacotherapies, which help manage the symptoms and signs of bladder dysfunction, but do not promote recovery of function. Spinal cord epidural stimulation (scES) has shown clinical effectiveness for the restoration of voluntary and reflex control of the lower urinary tract. The objective of this functional mapping study was to identify personalized neuromodulation with selective cohorts (anode and cathode assignment, pulse width, frequency, and amplitude) interleaved for specific scES configurations that promoted optimal neural control of both urinary storage (US-scES; bladder capacity at safe detrusor pressure without incontinence or triggering of autonomic dysreflexia) and timed detrusor emptying (UV-scES; high voiding efficiency within safe leak point pressures). Daily, at-home training with US-scES and UV-scES configurations were also conducted to evaluate the long-term effectiveness of lumbosacral neuromodulation for bladder function. Six participants (5 males, 1 female; 4 self-intermittent, 2 suprapubic; unable to voluntarily void) having motor complete SCI and implanted at lumbosacral spinal cord levels with a Medtronic 5-6-5 electrode array and neurostimulator, underwent bladder mapping experiments including cystometry with continuous measurements of blood pressure and heart rate using a non-invasive blood pressure monitoring system. Over a period of 4-6 weeks, mapping identified specific US-scES configurations for improved bladder compliance within the recommended International Continence Society guidelines (detrusor filling pressure <10 cmH_2O up to target capacity range of 400 - 450 mL for intermittent catheterization and > 300 mL for those using suprapubic catheters) as well as including cohorts targeted to maintain blood pressure below hypertensive values. All participants were able to

initiate voluntary voiding in the presence of UV-scES, regaining up to 70% maximum voiding efficiency. Lumbosacral neuromodulation with personalized mapping can be used to simultaneously and safely control urinary continence and modulate timely emptying while managing distention-associated blood pressure dysregulation. Importantly, these initial findings reveal the complex dynamics and interplay between autonomic systems that is being integrated and regulated within the spinal cord below the level of SCI.

Disclosures: A. Herrity: None. **S. Aslan:** None. **S. Mesbah:** None. **B. Ugiliweneza:** None. **A. Mohamed:** None. **C. Hubscher:** None. **S. Harkema:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.09

Topic: F.07. Autonomic Regulation

Support: SPARC OT2OD028191

Title: Quantifying anal sphincter recruitment during clinical pudendal nerve stimulation

Authors: *A. LAGUNAS[1], P.-J. CHEN[1], L. RUIZ PEREZ[1], A. U. KOTKAR[1], S. LEMPKA[1], P. GUPTA[2], T. BRUNS[1];
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Abstract: Overactive bladder and urinary retention have a negative impact on quality of life for millions of Americans. Unfortunately, as the population continues to age these problems will become even more widespread. Electrical stimulation of the pudendal nerve is a promising off-label treatment for bladder dysfunction. The goal of this study is to investigate the effects of differing pudendal nerve stimulation parameters on the evoked anal sphincter muscle response in human subjects. During the electrode implant surgery, the stimulator lead is placed adjacent to the pudendal nerve, which innervates the urethra and the anal sphincter. Needle electrodes placed on either side of the anus are used to record the anal sphincter electromyogram (EMG). Each lead electrode is stimulated at and above the EMG activation threshold at 3 Hz monopolar, with up to three different pulse widths: 60 μs, 210 μs, and 450 μs. For each tested set of stimulation parameters, the area under the curve (AUC) for ten rectified and averaged EMG responses to stimulation pulses were used to quantify muscle recruitment. Data from 4 patients (all female) are analyzed so far, in this on-going clinical study. The non-normally distributed AUC measures were square-root transformed to reduce the distribution skewness prior to linear regression analyses. Across all trials there was a slowly increasing linear relationship between AUC and stimulation amplitude (slope = 0.17, R^2 = 0.27, p = 1.46e-5) that varied slightly between the 60 μs (slope = 0.19, R^2 = 0.20, p = 0.048), 210 μs (slope = 0.21, R^2 = 0.34, p = 3.33e-5), and 450 μs

(slope = 0.27, R^2 = 0.33, p = 0.0055) pulse width trials. Overall, there is an increasing trend in the sphincter response as more stimulation current is applied across the stimulation amplitude range used (0.5-10 mA). While the linear fit slopes are similar, our preliminary results show trials with longer pulse widths evoke a larger muscle response over all applied currents. Further data collection and analysis may reveal additional insights into how varying stimulation parameters can help improve the effectiveness of pudendal nerve stimulation.

Disclosures: A. Lagunas: None. **P. Chen:** None. **P. Gupta:** None. **T. Bruns:** None.

Poster

P629. Renal, Urinary, and Bowel Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P629.10

Topic: F.07. Autonomic Regulation

Support: NIH SPARC program
OT2OD023873
OT2OD024899

Title: Sparc - catheter-free measurement of bowel activity using wireless intracolonic sensor

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Abstract: Background & Aims: The neurological mechanisms contributing to bowel dysfunction are not yet fully understood, in part because of the slow actions of the bowel and the limitations in wireless gut manometry systems. To enable multimodal data collection with a route to endoscopic implantation, we developed the Colonic Monitor of Conscious Activity (ColoMOCA). After implant, the ColoMOCA wirelessly transmits colonic pressures and electrical properties of stool over multiple days. We evaluated the accuracy of the ColoMOCA acutely in conscious swine, and the feasibility of characterizing bowel activity in awake, untethered swine over several days. **Methods:** The ColoMOCA (85 mm long, 8 mm diameter) included pressure-sensing electronics, electrodes for measuring stool salinity, and wirelessly transmitted data. The ColoMOCA measured pressure from 2 locations 63 mm apart, and measured stool conductivity in 3 overlapping regions.ColoMOCAs were tested in N=3 minipigs chronically and N=1 pig acutely. Acute insertions in conscious restrained animals included micro-tip pressure catheters alongside the ColoMOCA. Data were recorded acutely in 3 sessions.

Chronic ColoMOCAs were sutured to the colon luminal-wall through laparotomy. Daily recordings of up to 2 hours of colonic activity began the day after implantation. Colonic pressures and electrode readings were made with animals conscious and untethered. Data were received by a wireless radio worn by the animal. During recordings each animal ate food, walked, urinated, and defecated freely. Recorded pressure data were analyzed in MATLAB. The amplitude, duration, and periods between rhythmic peaks in colonic pressure waveforms were calculated. Statistical analysis was performed in JASP. Data from implanted animals were quantitatively and qualitatively analyzed. **Results**: 100 minutes of ColoMOCA data with concomitant micro-tip catheter measurements were obtained. Statistical analysis confirmed accuracy between ColoMOCA and reference pressure sensor data. In the chronic study, all animals recovered well and showed no signs of distress after 24 hrs. Implanted ColoMOCAs remained patent and did not obstruct stool for 6-7 days of implantation. 435 minutes of ambulatory, catheter-free colon pressure and conductivity data were recorded and analyzed. As in the acute case, implanted sensors showed a significant difference in contraction amplitudes between proximal and distal sensors. **Conclusion**: Small wireless sensors can remain in the bowel for at least 7 days, permitting catheter-free untethered recordings of colonic activity. These sensors will improve neurophysiological research of the bowel.

Disclosures: S. Majerus: None. **Y. Hacohen:** None. **B. Hanzlicek:** None. **A. Rietsch:** None. **J. Mrowca:** None. **A. Smiley:** None. **Y. Wang:** None. **W. Liu:** None. **M. Larauche:** None. **M. Mulugeta:** None. **M. Damaser:** None. **D.J. Bourbeau:** None.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.01

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics

Title: Psilocybin reduces impulsive decision-making without affecting motivation

Authors: *B. F. ROBERTS[1], J. D. CROWDER[3], S. J. LOBERGER[1], J. HINEGARDNER-HENDRICKS[1], M. PALUMBO[4], J. A. JONES[2], M. S. MCMURRAY[1];
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Abstract: Substance abuse and gambling disorders are strongly associated with increased preference for risky rewards and reductions in impulse control, resulting in harmful consequences. Although these disorders are highly prevalent, there are limited treatments available. Previous research has indicated clinical benefits of psilocybin, but its role in impulsive

decision-making has not been established. The serotonergic systems affected by psilocybin have established roles in decision-making, suggesting it may have significant effects.Therefore, this study investigated whether psilocybin impacts decision-making in probability and delay discounting tasks in male and female rats. In the probability discounting task, subjects chose between a certain, small reward and a risky, large reward that occurred with declining probability across days of testing. In the delay discounting task, animals chose between a small, immediate reward or a large reward delivered after increasingly longer delays across days of testing. Animals were administered 1mg/kg psilocybin via gavage, 15 minutes before each day's behavioral testing. Preliminary results suggest psilocybin decreases preference for the large risky reward in the probabilistic discounting task and increases preference for the larger delayed reward in the delayed discounting task. To determine if these effects are related to changes in motivation, subjects were tested on a progressive ratio task, in which the number of lever presses required to obtain reward increases with each subsequent reward. We found no effects of psilocybin on total responses or breakpoints. This pattern of results suggests that psilocybin may have utility as a novel therapeutic for gambling disorder and other impulse control disorders, such as addiction and ADHD.

Disclosures: B.F. Roberts: None. **J.D. Crowder:** None. **S.J. Loberger:** None. **J. Hinegardner-Hendricks:** None. **M. Palumbo:** None. **J.A. Jones:** A. Employment/Salary (full or part-time):; PsyBio Therapeutics. 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics. **M.S. McMurray:** 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.; PsyBio Therapeutics.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.02

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics

Title: Effects of a novel potential antidepressant norbaeocystin

Authors: *S. J. LOBERGER[1], J. D. CROWDER[3], *J. HINEGARDNER-HENDRICKS[1], J. A. JONES[2], M. S. MCMURRAY[1];

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Abstract: Clinical trials on the use of psilocybin to treat depression are promising, but still in preliminary stages. Additionally, they are confounded by the presence of hallucinations, which may not be necessary for its clinical effectiveness but create significant challenges in experimental design. Thus, there is a need to determine if other tryptamines have similar molecular targets, but do not cause hallucinations. Our research group recently identified a novel E. coli-based method for synthesizing norbaeocystin, a minor metabolite of psilocybin present in "magic" mushrooms. When administered alone, norbaeocystin did not cause head twitch responses in rats at any dose, implying it may not cause hallucinations. However, norbaeocystin did enhance the number of head twitch responses caused by psilocybin, implying that it may bind with overlapping molecular targets. Therefore, in this study we determined if norbaeocystin alone or combined with psilocybin has potential as an antidepressant using the sucrose preference and force swim tests. Preliminary results suggest that norbaeocystin alone causes a moderate decrease in immobility during forced swim and a moderate increase in sucrose preference. Additionally, when combined with psilocybin, the magnitude of effect in both tasks increases. Combined, these results suggest norbaeocystin may have strong potential as an antidepressant, alone or when combined with psilocybin. Future studies will validate these effects in other behavioral models and determine the mechanisms of these effects and drug interactions. Additionally, we will evaluate the potential therapeutic efficacy of other tryptamines present in "magic" mushrooms.

Disclosures: S.J. Loberger: None. **J.D. Crowder:** None. **J. Hinegardner-Hendricks:** None. **J.A. Jones:** A. Employment/Salary (full or part-time):; PsyBio Therapeutics. 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics. **M.S. McMurray:** 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.; PsyBio Therapeutics.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.03

Topic: G.05. Mood Disorders

Support: PSYBIO Therapeutics Research Grant

Title: Aeruginascin and norbaeocystin enhance the psychoactive effects of psilocybin in the rat

Authors: *J. D. HINEGARDNER[1], N. ANAS[2], J. CROWDER[1], S. LOBERGER[1], J. A. JONES[3], M. S. MCMURRAY[1];
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Abstract: Psilocybin has been shown to have numerous potential clinical benefits, and in the near future it could be used to treat a wide variety of human disease processes. However, other tryptamines present in "magic" mushrooms may have similar therapeutic effects without causing hallucinations, or may interact with psilocybin to enhance its therapeutic effect. Our lab has previously shown that norbaeocystin, one such tryptamine, interacts with psilocybin to enhance rodent head twitch responses, a metric of 5HT2A activation. Other tryptamines may work similarly, including aeruginascin, a compound recently synthesized by our research group. To test this hypothesis, we administered aeruginascin to rats via gavage, and compared its effect alone and in combination with psilocybin, to the effects of norbaeocystin alone and in combination with psilocybin. We found that like norbaeocystin, aeruginascin also enhances the number of head twitches caused by psilocybin, but causes no increase in head twitches when administered alone. To determine potential mechanisms of action, all compounds were screened by the NIMH Psychoactive Drug Screening Program, which found that norbaeocystin and aeruginascin have weak affinities for the 5HT2A receptor, but show stronger affinities for other serotonin receptor types. These data suggest that when administered concurrently with psilocybin, competitive binding for non-5HT2A targets may cause psilocybin to preferentially bind to 5HT2A receptors, enhancing the behavioral effects of psilocybin. Future studies will determine if norbaeocystin and aeruginascin have antidepressant and anxiolytic effects, either alone or in combination with psilocybin.

Disclosures: J.D. Hinegardner: None. **N. Anas:** None. **J. Crowder:** None. **S. Loberger:** None. **J.A. Jones:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics. **M.S. McMurray:** 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.; PsyBio Therapeutics.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.04

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics

Title: Oral administration of psilocybin and/or norbaeocystin cause persistent effects on the gut microbiome

Authors: *N. ANAS[1], J. HINEGARDNER-HENDRICKS[1], F. XU[2], H. SHI[2], J. A. JONES[3], M. S. MCMURRAY[1];
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Abstract: Recent clinical studies suggest that psilocybin may have antidepressant potential, although the mechanisms of these effects remain elusive. While significant progress has been made in understanding the brain systems responsible for depression, and their overlap with psilocybin's effects, a growing body of literature has also highlighted the importance of the gut-brain axis in mental health. Alterations to the gut microbiome have been shown to cause and maintain a variety of mental health disorders. Many gut bacteria express serotonin receptors and could be affected by psilocybin. Therefore, psilocybin may exert its therapeutic effects by altering the gut microbiome, similarly to other fast-acting antidepressants (e.g. ketamine). In this study, we determined if oral psilocybin treatment alters bacterial concentrations in the gut. Additionally, we determined if action on the 5-HT2A receptor was necessary for these effects by comparing psilocybin's effects with those of norbaeocystin, a minor metabolite of psilocybin with poor affinity for the 5-HT2A receptor. To accomplish these studies, we gavaged a variety of doses of psilocybin and norbaeocystin into male rats, both alone and in combination, and then compared bacterial concentrations in fecal samples collected at a variety of timepoints for each of the doses administered. We found that psilocybin and norbaeocystin both caused pronounced changes in gut microbiome that persisted up to 3 weeks following treatment, and that these effects were amplified when animals were given both drugs simultaneously. Specifically, we saw dose-dependent increases in the concentration of *Lactobacillus* and *Turicibacter*, paired with decreased concentrations of *Ruminococcus* after psilocybin and/or norbaeocystin administration, similar to the effects seen after ketamine. These effects demonstrate that psilocybin and other tryptamines may exert their therapeutic effects through non-5-HT2A receptor dependent alterations to the gut microbiome. These results have significant implications on our understanding of the mechanisms of these drugs, and imply that even isoforms of these compounds that cannot cross the blood brain barrier may have therapeutic utility.

Disclosures: N. Anas: None. **J. Hinegardner-Hendricks:** None. **F. Xu:** None. **H. Shi:** None. **J.A. Jones:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics. **M.S. McMurray:** 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.; PsyBio Therapeutics.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.05

Topic: G.05. Mood Disorders

Title: Effect of cannabidiol (CBD) in two animal models of depression

Authors: L. M. A. ZERMEÑO-FLORES[1], E. J. ROBLES-SIERRA[1], A. M. VILLARREAL-ZUÑIGA[1], F. D. ANCER-LEAL[2], F. J. ALMAGUER-DE AVILA[2], D. MATINEZ-CHAPOY[2], F. J. CRUZ-ARROYO[2], D. MOTIEL-CONDADO[1], A. C. GONZALEZ-HORTA[1], M. F. BERMUDEZ-DE LEON[3], *B. GONZALEZ-HERNANDEZ[1];
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Abstract: The world health organization estimates that there are currently 350 million people suffering from major depressive disorder. This is characterized by dysphoric mood and among the most serious consequences suiciding can became an option, apart from the social and economic burden it has. Although there are a lot of antidepressants, it is considered that 30% of patients do not respond to the treatment, in addition they are multiple side effects associated. That is why we are exploring a new therapeutic alternative such as the *Cannabis* plant that has about 500 compounds from these, 60 are considered psychoactive and one of the most studied is *Cannabidiol* (CBD), because it lacks in psychotropic properties and is well tolerated. This activates the endocanabinoid system, which participate in the regulation of mood and therefore in depressive processes. The objective of this work is to study the effect of CBD on acute and chronic depression in an animal model. Male and female Wistar rats of 210-260gr were used. The acute model of depression we used was the forced swimming test combined with a "time-sampling" analysis, which consists in forcing the rats to swim individually in two occasions: 1) A pre-session of 15 minutes and 24 hours later, 2) An experimental session of 5 minutes, in which they are three main behaviors that are recorded in intervals of 5 seconds: scaling, swimming and immobility. For chronic depression, the unpredictable chronic stress model (CUS) was used, a 4-week stressor regimen, in which each of the seven stressors (physical restrain, cage tilted, wet bedding, nip pinch, overcrowding, water and food deprivation), was applicated once a week, alternatively switching the test for each group, every day. At the end, the depression levels were quantified using the forced swim test. In the acute model, the single application of CBD (30mg / kg IP) decreased immobility in females, similar results we have as

we applied imipramine (15mg / Kg IP; positive control) having a very significant result in males. In the chronic model, the application of CBD (30mg / kg IP), during the last 5 days of CUS, showed no difference with respect to the result we have from the acute application in females and in males it shows an effect with a with a better trend than imipramine. These results indicate that CBD has an antidepressant effect in both models but shows a differential profile depending on the gender and the degree of depression, being the same in females regardless of the degree, but better antidepressant in males in the acute model.

Disclosures: L.M.A. Zermeño-Flores: None. **E.J. Robles-Sierra:** None. **A.M. Villarreal-Zuñiga:** None. **F.D. Ancer-Leal:** None. **F.J. Almaguer-De Avila:** None. **D. Matinez-Chapoy:** None. **F.J. Cruz-Arroyo:** None. **D. Motiel-Condado:** None. **A.C. Gonzalez-Horta:** None. **M.F. Bermudez-De Leon:** None. **B. Gonzalez-Hernandez:** None.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.06

Topic: G.05. Mood Disorders

Support: CoMRAD - 422467
CoMRAD - 424223

Title: Lithium Orotate: A Superior Option for Lithium Therapy?

Authors: *A. G. PACHOLKO[1], L. K. BEKAR[2];
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Abstract: Lithium carbonate (Li_2CO_3) is a mainstay treatment option for the prevention of mood-episode recurrences in bipolar disorder (BD). However, Li_2CO_3 is not without its faults, specifically a narrow therapeutic index that is associated with common thyroid and renal complications; consequently, lithium-based therapies are plagued by high rates of patient non-compliance. Lithium orotate (LiOr) is an alternative compound that is suggested to possess uptake properties that would allow for the use of markedly reduced dosages in treatment regimens, which would theoretically mitigate lithium-associated toxicity concerns and improve treatment compliance.
As a first step in exploring LiOr as a replacement for traditional lithium compounds in therapy, we established dose response curves for a range of LiOr, Li_2CO_3, and lithium chloride (LiCl) concentrations (0, 1, 1.5, 2.5, 5, 10 and 15 mg/kg for LiOr; 0, 1, 5, 10, 15, 20 and 25 mg/kg for Li_2CO_3/LiCl; all concentrations represent elemental lithium) following a single injection in both male and female wild-type mice using an amphetamine-induced hyperlocomotion (AIH) model

(3 mg/kg *d*-amphetamine). AIH is mania-mimetic and sensitive to a dose-dependent blockade via pre-treatment with lithium. Lithium was injected intraperitoneally (IP) 30 minutes prior to IP administration of *d*-amphetamine. Animals were recorded for 120 minutes in an open field and scored for total locomotion. Data was analyzed offline using Ethovision 11.x and Prism software. Application of *d*-amphetamine elevated locomotor activity chiefly within the 5-35- and 70-120-minute time bins post-injection, with the 70-120-minute window representing the peak period of hyperlocomotion. LiOr, but not LiCl/Li_2CO_3, elicited attenuation of AIH between minutes 5-35 at concentrations of 10 mg/kg or greater. For minutes 70-120 (the peak period of locomotor activity), a partial block of AIH was maintained by LiCl and Li_2CO_3 doses of 15 mg/kg or greater in males and 20 mg/kg or greater in females. In contrast, a complete blockade was observed for LiOr concentrations equal to or exceeding 2.5 mg/kg or 1.5 mg/kg in males and females, respectively, indicating both improved efficacy and potency relative to LiCl/Li_2CO_3. In sum, LiOr demonstrates superior efficacy to LiCl and Li_2CO_3 in the attenuation of the mania-mimetic AIH in both male and female wild-type mice, as evidenced by reduced dosage requirements and an enhanced strength of blockade at equivalent concentrations.

Disclosures: A.G. Pacholko: None. **L.K. Bekar:** None.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.07

Topic: G.05. Mood Disorders

Support: HDRF Grant RGA-13-003

Title: Contribution of adult born granule cells to the mechanism of action of electroconvulsive stimulation

Authors: *J. CASTELLO[1,2], V. LUNA[1,2,3], R. HEN[1,2,3];
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Abstract: Electroconvulsive therapy (ECT) is a safe, effective treatment with a fast onset of action in patients that suffer from treatment-resistant depression. However, the mechanism of action of ECT has not been elucidated. In the dentate gyrus (DG) of the hippocampus, ECT increases the production of adult born granule cells (abGCs), through a process termed adult hippocampal neurogenesis. We have successfully established a mouse model of ECT, named Electroconvulsive stimulation (ECS) and shown that abGCs are required for the behavioral effects of ECS. In this model, ECS stimulates the production of abGCs, increases the number of presynaptic boutons coming from abGCs in the molecular layer of the dentate gyrus and

decreases c-Fos activation.
In addition, optogenetic stimulation of abGCs induce an inhibitory current in mature granule cells mediated through mGluR2 receptors in ECS treated animals. Thus, our overarching hypothesis is that ECS, through stimulation of neurogenesis, incrases the number of presynaptic boutons from abGCs which lead to the activation of postsynaptic mGluR2 receptors on mature granule cells. We propose that the resulting inhibition of the dentate gyrus is responsible for the antidepressant-like effects of ECS.

Disclosures: J. Castello: None. **V. Luna:** None. **R. Hen:** None.

Poster

P710. Animal Models of Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P710.08

Topic: G.05. Mood Disorders

Support: 1R15MH11416101A1

Title: Behavioral Effects and Neurobiological Mechanisms of 3-Aminobenzimide ina Rodent Model of Chronic Psychological Stress

Authors: *L. J. WILLS[1], L. D. PEETERS[2], H. WANG-HEATON[2], M. J. CHANDLEY[2], G. A. ORDWAY[2], R. W. BROWN[2];
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Abstract: Stress-related disorders are a leading cause of disability worldwide. Antidepressant drugs are commonly used in the treatment of stress-related disorders, but patient estimates suggest that the rate of treatment resistance to antidepressants are over 30%. Since antidepressant drugs are modulators of monoamine transmission, the high level of treatment failure indicates that monoamine disruption is not the sole factor in the etiology of these disorders. Our lab has shown an increase in the expression of poly (ADP ribose) polymerase-1 (PARP1) in the white matter of the prefrontal cortex of brain donors who had major depressive disorder (MDD) at the time of death as compared to psychiatrically normal brain donors. To investigate the potential role of PARP1 in stress-induced behavioral disruption, the present study used a rodent "double-hit" model consisting of exposure to social defeat stress (SDS) and chronic unpredictable stress (CUS) to induce a state of chronic psychological distress. Male Sprague-Dawley rats were either in a no-stress group or were exposed to SDS and CUS. Stressed animals were administered with either a PARP-inhibitor, 3-aminobenzamide (3-AB; 40 mg/kg; s.c.); the selective serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine (FLX; 10 mg/kg; i.p.); or saline each day prior to exposure to stressors. Stressors were administered over a 10-day period. SDS was implemented by introducing a male "intruder" rat into a male "aggressor" rat's cage, at which

point the “aggressor” established dominance over the “intruder”. There were five stressors used for CUS, including a 30-min restraint, 1h shaking and crowding, 10-min cold water swim, 15-min warm water swim, and a 24h cage tip which were randomly administered twice to animals during the 10-day period. To assess responsiveness to rewarding stimuli, we conducted the sucrose preference task on experimental days 8-10 during the first two-hours of the animal’s dark cycle. A social interaction test was conducted on day 11 to measure anxiolytic behavior in the rats. Stressed rats that were chronically administered 3-AB exhibited a significantly increased sucrose preference and social interaction times when compared to all other groups, and FLX failed to. We investigated PARP activation and protein expression in the PFC using immunohistochemistry and ELISAs, IBA-1 expression in PFC microglial cells, and cytokine levels in hippocampal tissue using ELISAs, which will be presented. These findings elucidate the impact and mechanism of PARP pertaining to chronic stress and the novel use of PARP-inhibitors as therapeutic alternatives for the treatment of stress-related disorders.

Disclosures: L.J. Wills: None. **L.D. Peeters:** None. **H. Wang-Heaton:** None. **M.J. Chandley:** None. **G.A. Ordway:** None. **R.W. Brown:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA 5R01DA047441-02

Title: Nr4a2 dominant negative in medial habenula cholinergic neurons attenuates reinstatement of cocaine-self administration in mice

Authors: *J. E. CHILDS[1,2], S. MORABITO[1], S. DAS[1], C. SANTELLI[1], V. ALIZO VERA[1], V. PHAM[1], D. MATHEOS[1], V. SWARUP[2], M. A. WOOD[3];
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Abstract: Substance use disorder is complicated by a lifelong risk of relapse. Relapse vulnerability is programed partly during drug use, when the action of drugs of abuse in the reward circuit enables the formation of abnormally strong context/reward memories. These memory processes underlie common relapse triggers such as exposure to drug-associated cues or environments and can be extremely long lasting. This persistence may be attributed to epigenetics (i.e. modulation of gene expression that occurs through altered chromatin structure without fundamental changes to the DNA sequence itself), which has been shown to establish stable changes in cell function, and in turn changes in behavioral outcomes. Recent studies have begun to implicate the medial habenula (MHb) in cocaine-associated behaviors, yet the role of

the MHb in regulating reinstatement of cocaine self-administration remains largely unknown. Recent work in the lab has identified the histone deacetylase 3 (HDAC3; a powerful epigenetic regulator of gene expression) target gene, *Nr4a2*, as an important regulator of cocaine-associated behavior. NR4A2 is a transcription factor that regulates aspects of dopamine signaling during development, and is densely expressed in the MHb. To study the effect of MHb *Nr4a2* on relapse to drug-seeking behavior, we expressed the dominant negative form of NR4A2 (NURR2C) in the cholinergic neurons of the medial habenula in ChAT-Cre mice. Then, animals were trained to self-administer cocaine. To facilitate reinstatement behavior, we used an incubation of craving model wherein after self-administration, animals were given 30 days of homecage withdrawal. On the last day of testing, animals were extinguished for 5 hours, and were then re-exposed to drug-associated cues to drive a cued reinstatement. Control animals expressed normal reinstatement compared to NURR2C animals, which had reduced reinstatement. To develop a more in-depth understanding of the MHb response to reinstatement of cocaine-seeking, we next used single nuclei RNA sequencing to look for changes in cell type proportions, gene expression and gene networks both as a result of behavioral reinstatement and as a result of NR4A2 manipulations. In this study we show that NR4A2 in the MHb is a powerful regulator of reinstatement of cocaine-seeking behavior, and we identify Nr4a2-dependent mechanisms in the MHb related to reinstatement behavior.

Disclosures: J.E. Childs: None. **S. Morabito:** None. **S. Das:** None. **C. Santelli:** None. **V. Alizo Vera:** None. **V. Pham:** None. **D. Matheos:** None. **V. Swarup:** None. **M.A. Wood:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.02

Topic: G.09. Drugs of Abuse and Addiction

Support: DA051686
DA048562

Title: Interaction of kappa opioid receptor activation and dopaminergic signaling in the nucleus accumbens core mediate escalated cocaine intake

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Abstract: Alterations of dopaminergic signaling are hypothesized to be associated with several aspects of drug addiction. Cues that are repeatedly paired with delivery of drugs of abuse can become conditioned stimuli (CS) and are capable of driving dopamine (DA) release in the nucleus accumbens core (NAc). Recent work in our lab has demonstrated that an attenuation of CS driven dopaminergic signaling in the NAc during drug taking is correlated with escalated consumption of cocaine. Systemic administration of L-DOPA restored this neurochemical changed and returned drug consumption to pre-escalated levels. More recently, we found that precise increases in dopamine transmission during the CS paired with drug delivery decrease drug consumption. We accomplished this by injecting a viral vector that expresses ChR2 under the control of the CaMKIIα promoter, bilaterally into the ventral tegmental area, and implanted optic fibers in the NAc of male Wistar rats. Photostimulation (6 pulses, 30 Hz) paired with response-contingent drug cues decreased drug consumption in animals that escalate daily drug intake ($p<0.01$), indicating that low phasic DA drives escalation of consumption. Thus, preventing the attenuation of dopamine transmission in the NAc would inhibit the development of escalated cocaine consumption. One potential mechanism promoting the decrement of dopamine signaling is dynorphin via action on Kappa opioid receptors (KOR). To test this, we locally injected the KOR antagonist, norbinaltorphimine (norBNI) into the NAc. Animals treated with norBNI had no significant increase in drug intake across weeks ($p>0.05$), whereas animals treated with vehicle showed significant escalation ($p<0.05$). To further assess which population of KORs in the NAc is contributing to the effect on drug-consumption seen with norBNI we utilized CRISPR/SaCas9 to induce mutagenesis of KOR within the NAc. SaCas9 was packaged into an AAV1 viral vector and injected into either the NAc, to target cell bodies, or into the VTA, to inactivate KOR on DA terminals. This experiment revealed that inactivation of KOR on DA terminals led to a decrease in overall drug consumption ($p<0.05$) and blocked the development of escalation ($p>0.05$), whereas animals with KOR mutagenesis on cell bodies within the NAc still presented significant escalation of intake ($p<0.05$), similar to controls. These data support our proposed model that NAc KOR activation attenuates response-contingent CS dopaminergic signaling to promote escalated cocaine consumption. Development of KOR antagonists as a pharmacological therapy may have harm-reduction benefits for persons living with addiction.

Disclosures: R.D. Farero: None. **L. Gordon-Fennell:** None. **L.M. Burgeno:** None. **J. McAleer:** None. **A. Asarch:** None. **A.D. Abraham:** None. **N.L. Murray:** None. **C.I. Chavkin:** None. **L.S. Zweifel:** None. **P.E. Phillips:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: Involvement of endogenous ghrelin on the maintenance and reinstatement of cocaine addiction-like behaviors: a role of adrenergic action at the peripheralβ1 receptors

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Abstract: Involvement of endogenous ghrelin on the maintenance and reinstatement of cocaine addiction-like behaviors: a role of adrenergic action at peripheral β1 receptors
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ABSTRACT
Ghrelin, an orexigenic hormone secreted primarily from endocrine cells located primarily in the stomach, and gut, has been recently found not only to stimulate in its acylated form, mesolimbic dopamine transmission and modulate food reward, also to be involved in the regulation of the rewarding effects of most abused drugs - including alcohol, opioids and psychostimulants as tested using classical place conditioning. In this study, we systematically investigated the responses of ghrelin in bloodstream to cocaine self-administration and to systemic injection of cocaine, and the effects of ghrelin receptor antagonism on cocaine-related behaviors using the selective ghrelin receptor antagonist JMV2959 in rats. The potential involvement of adrenergic action at peripheral β1 receptors was explored by pretreatment with atenolol, a peripherally active restricted β1 receptor antagonist. We found that both cocaine self-administration (1 mg/kg /infusion) and i.p. injection of cocaine (10 mg/kg, i.p.) significantly elevated plasma ghrelin and des-acyl ghrelin levels. Cocaine self-administration had no effect on plasma LEAP2, a recently identified endogenous ghrelin receptor antagonist. Pretreatment of cocaine-trained rats with JMV2959 (0-6mg/kg, i.p.), a potent ghrelin receptor antagonist, dose-dependently inhibited cocaine self-administration and reinstatement of cocaine-seeking induced by cocaine (10 mg/kg, i.p.) in cocaine-trained and subsequently behaviorally extinguished rats. Pretreatment with atenolol (0-15 mg/kg, i.p.) also dose-dependently inhibited cocaine self-administration and cocaine-induced reinstatement. Atenolol at 15 mg/kg also blocked the elevation in plasma ghrelin induced by cocaine. Our findings reveal an important role of endogenous ghrelin in cocaine self-administration and reinstatement and indicate that activation of peripheral β1 adrenergic receptors may be an essential process for cocaine to elevate ghrelin and for ghrelin to mediate the rewarding and motivational effects of cocaine. Thus, manipulations of the ghrelin system may represent a feasible approach for psychostimulant addiction treatment.
Supported by funds from NIDA-IRP

Disclosures: M. Crissman: None. **S. Pari:** None. **Z. You:** None. **L. Leggio:** None. **E.L. Gardner:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.04

Topic: G.09. Drugs of Abuse and Addiction

Support: DA048651

Title: Abstinence from cocaine self-administration alters expression of circHomer1 in the nucleus accumbens shell.

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Abstract: Cocaine and other psychostimulants induce long lasting changes in mesolimbic 'reward' circuits of the brain that persist or emerge after cessation of substance use. These lingering changes include the neuronal plasticity that is thought to underlie the chronic relapsing nature of substance use disorders. Genes encoding proteins involved in neuronal plasticity can also generate circular RNAs (circRNAs), which are a group of stable, non-coding RNAs produced through back-splicing of pre-mRNAs. CircRNAs are highly expressed in neuronal tissues, specifically synapses; however, their role in the brain is not well understood. The *Homer1* gene encodes several proteins associated with cocaine-induced changes in postsynaptic plasticity, and generates the circRNA *circHomer1*. Preliminary evidence from environmental enrichment (EE) studies in our lab show that relative changes in *circHomer1* and its linear RNA counterpart *Homer1b* in the nucleus accumbens (NAc) shell may be involved in incentive motivation for cocaine. To further examine the relationship between *circHomer1* and drug motivation, we used different lengths of forced abstinence to vary the degree of motivation for cocaine. Male (n=14) and female (n=15) Sprague Dawley rats were trained to self-administer cocaine (0.75 mg/kg/infusion, IV) or were yoked saline controls while advancing through increasingly difficult schedules of reinforcement until they ultimately reached VR5, the highest level of difficulty to obtain the drug reward. Following either 1 or 21 days in forced abstinence, rats were given a 1-h cue reactivity test and were sacrificed immediately after. *CircHomer1* expression was then measured from crude synaptosomal fractions of NAc shell tissue punches using RT-qPCR. Lever pressing during the cue reactivity test was significantly greater in animals abstinent for 21 days, in support of the incubation of craving effect. Preliminary results show a decrease of *circHomer1* expression in male rats abstinent from cocaine for 21 days compared to 1 day of abstinence. However, no difference in *circHomer1* levels was seen in female rats regardless of abstinence length. These preliminary results suggest that *circHomer1* expression is inversely associated with the incubation of craving effect in male rats, but that sex differences may underlie *circHomer1* expression and subsequent modulation of synaptic plasticity.
Supported by DA048651

Disclosures: M.C. Johnson: None. **G.L. Powell:** None. **A. Vannan:** None. **M. Dell'Orco:** None. **N. Mellios:** None. **N. Perrone-Bizzozero:** None. **J.L. Neisewander:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Pairing extinction of cocaine-seeking with vagus nerve stimulation (VNS) reduces reinstatement via TrkB activation

Authors: *C. M. DRISKILL[1], A. J. PHENSY[1], S. R. RODRIGUEZ[1], K. L. LINDQUIST[1], J. F. MCGINTY[2], S. KROENER[1];
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Abstract: Cocaine induces neuroadaptations in the prefrontal cortex that contribute to drug-seeking and relapse. Vagus nerve stimulation (VNS) causes the release of several neuromodulators, including BDNF, which modulate cortical plasticity, and this plasticity can be used to increase learning and memory in both rats and humans. We have recently shown that VNS facilitates extinction learning and reduces cue-induced reinstatement in cocaine self-administering rats. Repeated exposure to cocaine and withdrawal can lower the levels of BDNF in the mPFC. Replacement of BDNF via a single infusion into the prelimbic cortex immediately after cocaine self-administration reduces relapse to cocaine-seeking after abstinence, as well as cue- and cocaine prime-induced reinstatement following extinction. Inhibiting TrkB BDNF receptors, or NMDA receptors blocks this effect, indicating an important interaction between glutamatergic synaptic activity and TrkB signaling in BDNF's suppressive effects on drug-seeking. Here we tested if VNS-induced BDNF release can modulate extinction and reinstatement of cocaine-seeking. VNS-treated rats showed elevated levels of BDNF (as measured via ELISA) in the mPFC after a single day of extinction. Systemic injections of the TrkB receptor antagonist ANA-12 during the 10d extinction period greatly reduced the effects of VNS on drug-seeking during cue-induced reinstatement. VNS-treated rats that received vehicle infusions made fewer lever presses during reinstatement when compared to sham-stimulated rats. In contrast, VNS-treated rats that received ANA-12 during extinction responded similar to Sham-stimulated rats. Following reinstatement rats were sacrificed for whole cell patch-clamp recordings to measure how VNS-induced BDNF affects glutamatergic transmission in the mPFC. Pyramidal neurons from Sham-stimulated rats animals showed reduced AMPA:NMDA ratios compared to drug-naïve animals, and treatment with VNS reversed this effect. AMPA:NMDA ratios in VNS+ANA-12-treated rats were similar to those in Sham-stimulated rats. In order to further test changes in glutamatergic synaptic transmission we recorded spontaneous EPSCs,

which at -70mV almost exclusively represent AMPAR currents, and measured changes in amplitude and frequency. Sham-stimulated rats showed a significant reduction in the average amplitude of sEPSCs compared to drug-naïve and VNS-treated rats, without changes in the frequency of sEPSCs. Taken together, these data suggest that cocaine-seeking alters AMPA currents in the mPFC and that VNS-induced BDNF release can reverse these changes to consolidate extinction learning and reduce reinstatement.

Disclosures: C.M. Driskill: None. **A.J. Phensy:** None. **S.R. Rodriguez:** None. **K.L. Lindquist:** None. **J.F. McGinty:** None. **S. Kroener:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R00DA037271

Title: Effect of systemic protein synthesis inhibition on reconsolidation of adolescent-formed drug-context associations

Authors: *A. N. HERRERA CHARPENTIER, B. R. CHO, D. I. OLEKANMA, C. A. REEVES, A. A. ARGUELLO;
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Abstract: Adolescents are at increased risk to develop substance use disorders and suffer from relapse throughout life due to the strong associative memories formed during drug use. Previous research has used adult rodent models of relapse to investigate the role of reconsolidation mechanisms in strengthening maladaptive drug memories. Specifically, systemic treatment with the protein synthesis inhibitor cycloheximide (CHX) after a brief re-exposure to a previous drug-related cue diminished relapse behavior. However, it is unknown whether adolescent-formed, drug-context associations can be modified to reduce risk of relapse. To answer this question, we used an abbreviated self-administration paradigm that allows us to manipulate reconsolidation and test subsequent relapse-like behavior during adolescence. Adolescent male Sprague-Dawley rats (P33) received jugular catheterization surgery and were taken through cocaine self-administration training (Coc-SA; distinct contextual environment, FR1 schedule of reinforcement, 2-hr session, 2x/day, minimum of 10 sessions) and extinction training (EXT; alternate context, 2-hr session, 2x/day, minimum of 8 sessions). After the last session of EXT, rats underwent a single memory reactivation (MR) session to retrieve cocaine context memories. During MR, rats were returned to the previous Coc-paired context for a 5min or 15min session, followed immediately with a subcutaneous injection of vehicle (VEH) or CHX. Rats went

through two additional EXT sessions followed by reinstatement tests in the Coc-paired context. During the test session, levers responses were not reinforced and therefore measured drug-seeking behavior. Adolescent rats displayed similar levels of reinstatement regardless of treatment (CHX/VEH) or length of retrieval (5 vs 15 min MR session). Our results suggest that weakening contextual cocaine memories with a systemic protein synthesis inhibitor does not impair relapse during adolescence. Future directions will assess whether these observed effects are due to the specific stage of development or type of drug-associated stimuli (i.e. adolescence vs adulthood; context vs cue). Understanding the mechanisms of reconsolidation that can be influenced from adolescence can help in the development of treatments for relapse.

Disclosures: A.N. Herrera Charpentier: None. **B.R. Cho:** None. **D.I. Olekanma:** None. **C.A. Reeves:** None. **A.A. Arguello:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Investigating the role of tachykinin 2 (Tac2)/neurokinin B (NkB) in medial habenula-dependent reinstatement

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Abstract: Recently, our lab discovered that a unique set of neurons in the ventral medial habenula (MHb) are sufficient to drive drug-induced reinstatement behavior using a cocaine conditioned place preference (CPP) task and cocaine self-administration (new, unpublished data). Activating these ventral MHb neurons resulted in complete reinstatement of drug seeking, even in the absence of re-exposure to the drug, demonstrating how important these neurons are for relapse-like behavior. A key open question is, what molecular mechanisms are responsible for these processes within these neurons? Our preliminary data suggest that TAC2 may be pivotal.*Tac2* belongs to the family of neuropeptides called Tachykinins. Tachykinins and their receptors are known to be involved in motivated behaviors and are found to be highly expressed in brain regions associated with reward-related behaviors such as the MHb. Indeed, *Tac2* is expressed in the ventral component of the MHb. Although very little is known about the function of TAC2 in the MHb, a recent study demonstrated that nicotine-dependent effects on the MHb are dependent on NK3R, the receptor used by TAC2 (Biasi, Mariella De, et al., 2016). Additionally, TAC2 was found to act locally on different brain regions coordinating a variety of behavioral consequences as a result of chronic social isolation stress (Zelikowsky et al., 2018). This data suggests there may be an important interaction between stress and stress-induced

reinstatement of drug seeking. We aimed to understand how *Tac2* expression is regulated during different cocaine-seeking behavioral phases. In order to do so, wild type mice underwent cocaine CPP, and expression of *tachykinin* family mRNA was examined using RT-qPCR following different phases of CPP. A trend towards increased *Tac2* in the MHb was observed following cocaine-primed reinstatement as compared to saline-primed reinstated animals. To determine whether *Tac2* is necessary to drive cocaine-seeking behaviors, siRNA smart pool against Tac2 will be bilaterally injected into brain regions with upregulated *Tac2* prior to CPP. Results from this investigation will provide a clearer understanding of the molecular basis of addictive behaviors.

Disclosures: V. Alizo Vera: None. **J. Childs:** None. **M.A. Wood:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA023281

Title: PPL-138 (BU10038): a bifunctional mu-NOP partial agonist that reduces cocaine taking and does not maintain self-administration behavior

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Abstract: In recent years, cocaine overdose deaths have been on the rise. Despite being a public health problem for decades, there are currently no medications approved for the treatment of cocaine use disorder (CUD). However, the development of compounds with bifunctional agonist activity at both mu-opioid and nociceptin opioid peptide (NOP) receptors has appeared as a potential pharmacotherapeutic strategy. Formerly known as BU10038, PPL-138 is one such compound that has partial agonist activity at both mu-opioid and NOP receptors. Here we show that PPL-138 effectively decreased the total number of cocaine infusions and burst-like intake in both male and female rats using an intermittent access (IntA) operant cocaine-self administration model, meant to closely mimic the binge pattern of consumption in human cocaine users. Doses of PPL-138 that effectively reduced parameters of cocaine self-administration had no effect on lever presses for food in an IntA model of food self-administration for either sex, although locomotor activity was increased in female but not male rats. J-113397, a selective antagonist of NOP receptors (5 mg/kg), did not impede the anti-cocaine effect of PPL-138 in contrast to naltrexone, a mu-opioid receptor antagonist (1 mg/kg), which prevented the decrease in cocaine

self-administration induced by PPL-138 treatment. Additionally, treatment with the mu-opioid receptor agonist morphine (1, 3, 10 mg/kg) dose-dependently reduced the measures of IntA cocaine self-administration. This suggests that the anti-cocaine properties of PPL-138 are mechanistically mediated by mu-opioid receptors. Finally, although PPL-138 initially appeared rewarding under our experimental conditions, self-administration of the compound was not maintained. These findings demonstrate that the approach of combining partial mu-opioid/NOP receptor activation successfully decreases cocaine intake, is endowed with low abuse potential, and thus may provide a possible method for treating cocaine use disorder.

Disclosures: A. Cippitelli: None. **M. Martinez:** None. **G. Zribi:** None. **G. Cami-Kobeci:** None. **S.M. Husbands:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Bath. **L. Toll:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phoenix PharmaLabs.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA DA011064

Title: 5-ht1b receptor agonist produces similar abstinence dependent modulation of motivation for cocaine in male and female rats

Authors: *S. SCOTT, B. RUSCITTI, T. CHAPMAN, R. GARCIA, G. POWELL, J. NEISEWANDER;
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Abstract: Previous findings from our lab and others indicate that serotonin 1B receptors ($5HT_{1B}$Rs) modulate cocaine self-administration (SA) in opposite directions depending on the phase of the addiction cycle in male rats. In particular, the $5HT_{1B}$R agonist, CP 94,253 (CP) enhances cocaine intake during *maintenance* of daily cocaine SA on a low effort fixed ratio (FR) and a high effort progressive ratio (PR) schedule of reinforcement in male rats, while attenuating cocaine intake as well as drug-seeking behavior following 21 days of protracted *abstinence*. In contrast to male rats, however, we recently discovered that CP attenuates cocaine intake on an FR5 schedule in female rats, regardless of abstinence. In this study, we compared CP effects across sexes on cocaine SA under a PR schedule of reinforcement and on cue-induced cocaine-seeking behavior after protracted abstinence. Male and female Sprague-Dawley rats were trained to self-administer 0.75 mg/kg, IV cocaine on an FR1 schedule of reinforcement for 3 hours/day.

Once reinforcement rates stabilized, rats transitioned to self-administering 0.375 mg/kg, IV cocaine on a PR schedule. After reaching stability once again, rats were repeatedly tested for the effects of CP (0, 3.2, 5.6 and 10 mg/kg, SC) given 15 minutes prior to the start of a session of cocaine SA on the PR schedule. To examine the effects of CP on cue reactivity, male and female rats with a history of SA were placed into abstinence for 21 or 60 days. Following abstinence, rats were tested twice after pretreated with vehicle prior to one test and CP (5.6 mg/kg, SC) 15 minutes prior to the other test, with order of vehicle and CP tests counterbalanced across rats. Test sessions lasted 2 h during which the cocaine-conditioned light and tone cues previously paired with cocaine infusions were delivered on an FR1 schedule without cocaine. Results indicate that CP (5.6 mg/kg) increases cocaine intake on the PR schedule regardless of sex in rats that have not experience prolong abstinence, but attenuates active and inactive lever responses in rats after 21 or 60 days abstinence. These findings suggest that regardless of sex, CP has opposing effects on motivation for cocaine depending on whether or not rats experience abstinence from cocaine. Thus, the 5-HT_{1B}R agonist may be a useful treatment for cocaine use disorder after a period of abstinence.
Supported by: NIDA DA011064

Disclosures: S. Scott: None. **B. Ruscitti:** None. **T. Chapman:** None. **R. Garcia:** None. **G. Powell:** None. **J. Neisewander:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-NIGMS 2R25GM082406
RCMI-Pilot Project NMHHD U54 MD007579
B.R.A.I.N. and M.A.G.I.C. Core. NIM-HD MD007579
Catalyzer Research Grants Program- CRG-2020-00114
NIH-NIDA Fellowship # 1F31DA053793-01

Title: Effects of chronic stress on cocaine seeking behavior after forced abstinence in male and female rats

Authors: *R. MORALES SILVA[1], U. GELPI-DOMINGUEZ[2], G. RODRIGUEZ-TORRES[1], J. PEREZ TORRES[1], Y. PEREZ PEREZ[1], M. T. SEPULVEDA ORENGO[1];
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Abstract: Generally, Post-Traumatic Stress Disorder (PTSD) and Substance Use Disorder (SUD) are studied independently in preclinical studies, although they can occur concurrently in

patients and in fact, PTSD patients exhibit higher rates of SUD. Exposure to stressful or traumatic events can lead to the development of PTSD, and stress has been associated with a higher probability of relapse. Previous studies have shown that modified single prolonged stress reduces cocaine self-administration in rats, as well as a reduced cue-induced reinstatement of cocaine seeking behavior, without any effects on acquisition and extinction of cocaine self-administration. These studies used a short-access cocaine self-administration paradigm, and the effects of chronic stress on extended-access cocaine self-administration remain to be studied. Our objective is to determine the effects of chronic stress on cocaine seeking behavior in male and female rats. We hypothesize that unescapable footshocks prior cocaine self-administration will increase cocaine seeking behavior in both sexes. To test this hypothesis, we used unescapable footshocks for a period of 5 days at an intensity of 0.50mA (presented randomly), followed by 6-hour sessions of extended-access cocaine self-administration for 10 days, and a 30 day forced abstinence period. Subsequently, we examined conditioned stimulus- and cocaine-induced cocaine-seeking behavior. Our data show that unescapable footshocks prior cocaine acquisition increases cocaine-primed memory retrieval after withdrawal in male and female rats. Interestingly, only female rats show an increase in cue-induced cocaine seeking behavior in the stress group, compared with the control group. Furthermore, preliminary data show that the stress group exhibits an increase in motivation for drug-seeking behavior in a fixed ratio 3 schedule of self-administration sessions, compared with control group. These results suggest that chronic stress prior cocaine exposure increases the reinforcing effects of cocaine after forced abstinence in male and female rats, as well as the compulsivity of cocaine seeking behavior.

Disclosures: R. Morales Silva: None. **U. Gelpi-Dominguez:** None. **G. Rodriguez-Torres:** None. **J. Perez Torres:** None. **Y. Perez Perez:** None. **M.T. Sepulveda Orengo:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA044308
NIH DA051551

Title: Granulocyte-macrophage colony-stimulating factoralters cocaine-induced behavioral responses and transcriptional regulation in the nucleus accumbens

Authors: *K. E. LUCERNE[1], C. D. TEAGUE[1], D. D. KIRALY[2];
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Abstract: Increasing evidence from recent years shows the gut microbiome markedly effects behavioral and neurobiological responses to cocaine. Here we investigate the potential role of the immune system as a mechanism underlying gut-brain communication in cocaine use. To this end, we performed serum multiplex analysis of circulating cytokines in mice with intact or depleted gut microbiomes after chronic cocaine or saline. This analysis identified granulocyte-macrophage colony-stimulating factor (GM-CSF) to be significantly increased by chronic cocaine only in animals with an intact gut microbiome. To examine whether GM-CSF regulates behavioral responses to cocaine, we performed a cocaine conditioned place preference (CPP) assay on mice with intact or depleted microbiomes, receiving daily injections of GM-CSF (10μg/kg) or vehicle. On this CPP test, GM-CSF treatment normalized cocaine preference in microbiome-depleted animals, and significantly attenuated cocaine preference in microbiome-intact animals. Furthermore, GM-CSF (10μg/kg) 30 minutes prior to an acute dose of cocaine (7.5mg/kg) attenuated cocaine-induced locomotion. Given the effect of GM-CSF on cocaine behavioral responses, we then sought to investigate the biological consequences of GM-CSF on cocaine-induced transcriptional responses in the NAc. To this end, we performed quantitative polymerase chain reaction (qPCR) and RNAscope *in-situ* hybridization to quantify GM-CSF receptor expression in the nucleus accumbens (NAc) following cocaine treatment. qPCR and RNAscope showed cell-type specific cocaine-induced increases in GM-CSF receptor expression in the NAc. Based upon this evidence for altered GM-CSF signaling in the NAc following cocaine, we then performed RNA-sequencing on NAc tissue from GM-CSF+cocaine treated animals. We found that treatment with GM-CSF+cocaine altered genes related to synaptic function in the NAc. Altogether, these data indicate GM-CSF participates in cocaine-induced behavioral and molecular plasticity, poising it as a novel neuroimmune signal in cocaine use.

Disclosures: K.E. Lucerne: None. **C.D. Teague:** None. **D.D. Kiraly:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.12

Topic: G.09. Drugs of Abuse and Addiction

Support: R01-DA031900

Title: Hypocretin Receptor 1 Antagonism Blocks Incubation of Cocaine Craving and Normalizes Nucleus Accumbens Adaptations

Authors: *P. J. CLARK, R. A. ESPAÑA;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Relapse remains a major obstacle to recovery from drug dependence. Across all major drugs of abuse, drug craving has been reported to intensify during periods of abstinence. This phenomenon-known as incubation of craving-is thought to promote drug seeking, thereby increasing the risk of relapse. The neurobiology that underlies drug craving is complex, but recent work has revealed that incubation of cocaine craving is linked to aberrant glutamate plasticity within the nucleus accumbens (NAc). While the mechanisms remain unclear, dopamine (DA) transmission in the NAc is posited to mediate the emergence of these adaptations. Furthermore, we and others show that NAc DA terminals exhibit dysfunction during abstinence from cocaine, including increased baseline uptake rate, enhanced sensitivity to cocaine, and increased phosphorylation of the DA transporter (pDAT). Hypocretin receptor 1 antagonists have been shown to decrease each of these metrics and consistently reduce motivation for cocaine. These features position hypocretin receptor 1 as a potential therapeutic target for the treatment of drug craving and its associated neuropathology. To examine the trajectory of NAc adaptations during abstinence from cocaine, we first trained rats to self-administer cocaine on an intermittent access (IntA) schedule of reinforcement followed by one week of abstinence. As expected, we found that IntA and abstinence produced robust incubation of cocaine craving. Then, using *ex vivo* fast scan cyclic voltammetry (FSCV), we saw that incubation of craving was tied to higher DA uptake rate in the NAc and enhanced DA terminal sensitivity to cocaine. In NAc tissue from the same animals we found increased pDAT levels as well as elevated NMDA receptor protein levels using Western blots. Next, we examined whether treatment with the hypocretin receptor 1 antagonist, RTIOX-276, early in abstinence prevents incubation of cocaine craving and reduces aberrant dopaminergic and glutamatergic adaptations in the NAc. We again trained animals to self-administer cocaine on an IntA schedule followed by one week of abstinence. On the first day of abstinence, rats were treated with a single injection of RTIOX-276 or vehicle. We found that a single RTIOX-276 treatment blocked incubation of cocaine craving. Ongoing experiments will use western blots and *ex vivo* FSCV to examine the functional and biochemical consequences of RTIOX-276 treatment. Together, these data suggest that antagonism of hypocretin receptor 1 early in abstinence from cocaine blocks incubation of cocaine craving and may normalize NAc adaptations.

Disclosures: P.J. Clark: None. **R.A. España:** None.

Poster

P748. Cocaine: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P748.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant R21 DA039393
NIH/NIDA Grant R01 DA037897

UPenn Undergraduate Fellowship for Neuroscience Research

Title: A novel role for amygdalar GLP-1 circuits in the reinstatement of cocaine-seeking behavior during withdrawal

Authors: *R. MERKEL, K. RAGNINI, R. HERMAN, Y. ZHANG, H. D. SCHMIDT; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Cocaine is a widely abused illicit drug and cocaine use disorder (CUD) is a major public health concern as nearly 1 million American adults met the diagnostic criteria for CUD in 2018. Preventing relapse and promoting long-term abstinence remain the greatest challenges for successfully treating CUD. Unfortunately, there are currently no FDA-approved pharmacotherapies to reduce the rate of cocaine relapse. Thus, further exploration of the neurological mechanisms underlying relapse is imperative to fill this gap in knowledge. An emerging literature indicates that activation of central glucagon-like peptide-1 receptors (GLP-1Rs) reduces the rewarding effects of cocaine and attenuates cocaine-taking during voluntary self-administration and -seeking during abstinence. However, the neural circuits mediating these effects are not fully described. The central nucleus of the amygdala (CeA) is necessary for drug-related learning and the reinstatement of cocaine-seeking behavior. We hypothesized that activation of GLP-1Rs in the CeA via direct administration of the GLP-1R agonist, exendin-4, would attenuate the reinstatement of cocaine-seeking behavior. Here, we show that intra-CeA administration of exendin-4 dose-dependently attenuated the ability of an acute priming injection of cocaine and drug-paired cues to reinstate cocaine seeking during withdrawal. To date, GLP-1R-expressing circuits in the amygdala have not been characterized. Neural tracing and fluorescent *in situ* hybridization techniques were used to determine if GLP-1R-expressing neurons in the CeA send monosynaptic projections to the bed nucleus of the stria terminalis (BNST). We hypothesized that the BNST is a primary downstream target of CeA GLP-1R-expressing neurons due to its role in withdrawal phenotypes. Preliminary data suggest that GLP-1Rs are expressed on a subset of neurons that project from the CeA to the BNST. Together, these findings establish a novel, functional role for CeA GLP-1R signaling in cocaine reinstatement and further support re-purposing GLP-1R agonists for treating CUD. Future studies will determine if, and quantify the extent to which, GLP-1Rs are expressed on GABA neurons that project from the CeA to the BNST. We also aim to explore how CeA GLP-1 signaling influences stress-induced reinstatement of cocaine seeking and withdrawal-induced anxiety-like behaviors. Given the critical role of the CeA in cocaine reinstatement and stress, it is possible that CeA GLP-1 signaling mediates drug reinforcement indirectly by attenuating stress responses that drive drug-seeking behaviors during withdrawal.

Disclosures: R. Merkel: None. **K. Ragnini:** None. **R. Herman:** None. **Y. Zhang:** None. **H.D. Schmidt:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.01

Topic: G.09. Drugs of Abuse and Addiction

Support: F31 DA053105
T32 DA041349
P50 DA006634

Title: Synaptic vesicle protein, synaptogyrin-3, alters dopamine system function and reduces cocaine self-administration.

Authors: *E. G. PECK[1], P. M. ESTAVE[2], S. R. JONES[2];
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Abstract: Synaptogyrin-3 (SYG3) is a synaptic vesicle protein highly expressed in dopamine-containing neurons that directly interacts with the dopamine transporter (DAT), suggesting a role in synaptic dopamine dynamics. We tested the hypothesis that chronic exposure to cocaine will disrupt SYG3 function, leading to alterations in DAT that drive subsequent excessive cocaine taking. Rats were trained to self-administer cocaine, and after successful acquisition, rats were tested on a progressive ratio (PR) schedule of reinforcement. Western blots indicated a significant positive correlation between relative SYG3 and DAT protein levels in the ventral tegmental area, and a significant negative correlation between SYG3 and PR breakpoint. We then overexpressed SYG3 in the VTA of naive rats to assess alterations in baseline behavior and terminal dopamine dynamics. Anxiety-like behavior was assessed using the novel open field test, elevated plus test, and social interaction test. Then, cocaine self-administration behavior was assessed using a long access schedule of reinforcement. In a separate cohort of rats, nucleus accumbens dopamine terminal function was assessed using *ex vivo* fast-scan cyclic voltammetry (FSCV). Overexpression of SYG3 in the VTA resulted in greater time spent in the center of the open field, reduced cocaine self-administration, as well as augmented dopamine release and reuptake kinetics in brain slices containing the nucleus accumbens. Together, these data suggest that SYG3 is a powerful modulator of dopamine kinetics and dopamine-related behavior, indicating this as a fruitful potential target for pharmacotherapeutics to treat cocaine use disorder.

Disclosures: E.G. Peck: None. **P.M. Estave:** None. **S.R. Jones:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: The role of Grin2c expression in PFC ensemble neurons that encode cocaine conditioned place preference in mice

Authors: *L. A. RAMSEY, K. E. SAVELL, R. MADANGOPAL, C. N. MILLER, C. T. RICHIE, F. J. RUBIO, B. T. HOPE;
NIDA IRP, Baltimore, MD

Abstract: Learned associations between rewards, such as food or drugs of abuse, and reward-predictive stimuli, are critical to motivated behavior. Associative learning is thought to be encoded by functional alterations within activity-dependent neuronal ensembles. The specific mechanism by which learned associations are encoded within activity-dependent ensembles remains unknown. To examine these drug-based learned associations, we used cocaine conditioned place preference (CPP) in mice. We performed immunohistochemistry for the immediate early gene Fos, which is a marker of strong neuronal activation. We found that the prelimbic cortex is strongly active during cocaine-paired context re-exposure. We then used a combination of fluorescence activated cell sorting (FACS) and subsequent qPCR, as well as RNAscope in situ hybridization, to identify genetic targets that distinguish Fos-positive ensemble neurons from Fos-negative neurons in the prefrontal cortex following context re-exposure. We found an upregulation in expression of Grin2c, which encodes the NR2C subunit of the NMDA receptor, primarily in activated neurons after context re-exposure. Future studies will use miRNA-mediated knockdown of Grin2c to examine its role in the acquisition and expression of cocaine CPP.

Disclosures: L.A. Ramsey: None. **K.E. Savell:** None. **R. Madangopal:** None. **C.N. Miller:** None. **C.T. Richie:** None. **F.J. Rubio:** None. **B.T. Hope:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant RO1 DA053743-01A1

Title: Reelin protein marks cocaine-sensitiveDrd1+medium spiny neurons and modulates the transcriptional and physiological response to dopamine

Authors: *K. L. BRIDA[1], R. A. PHILLIPS, III[1], N. DAVIS[1], K. MONTGOMERY[2], K. MAYNARD[2], K. MARTINOWICH[2], J. J. DAY[1];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Reelin protein marks cocaine-sensitive *Drd1+* medium spiny neurons and modulates the transcriptional and physiological response to dopamine

Kasey L. Brida[1], Robert A. Phillips III[1], M. Natalie Davis[1], Kelsey Montgomery[2], Kristen R. Maynard[2], Keri Martinowich[2], & Jeremy J. Day[11]Department of Neurobiology, University of Alabama at Birmingham, [2]Lieber Institute for Brain Development

Reelin, a large, secreted glycoprotein encoded by the gene *Reln*, is expressed at high levels in the adult striatum, hippocampus, and cerebellum. *Reln* expression plays a critical role in brain development and experience-dependent plasticity. While the role of reelin protein in neurodevelopment has been extensively studied, reelin's cellular and molecular role in the adult brain remains to be characterized, despite genetic links to neuropsychiatric disorders, such as psychostimulant abuse. To assess *Reln* mRNA distribution within specific cell types of the rat nucleus accumbens (NAc), we queried a recently described transcriptional atlas generated from single-nucleus RNA-seq of rat NAc tissue collected following acute cocaine exposure. This dataset demonstrated that *Reln* mRNA marks a population of cocaine-responsive *Drd-1+* medium spiny neurons (MSNs). These results were mirrored in postmortem human brain tissue, where multiplexed fluorescent in-situ hybridization showed enrichment of *RELN* mRNA in NAc *Drd1+* MSNs. We next designed a CRISPR sgRNA targeting the *Reln* promoter, allowing us to bidirectionally manipulate *Reln* mRNA and protein levels with CRISPR activation or CRISPR interference. Notably, CRISPR activation of *Reln* in rat primary striatal neuron cultures enhanced stimulus-dependent transcription of immediate early genes following dopamine stimulation. Likewise, knockdown of *Reln* blunted the dopamine-induced increases in MSN firing rate, without altering baseline electrophysiological properties. These results suggest that Reelin may contribute to dopamine-dependent transcriptional and physiological changes caused by cocaine. Ongoing studies are assessing the effects of CRISPR-mediated *Reln* manipulations on cocaine-induced behavioral responses.

Disclosures: K.L. Brida: None. **R.A. Phillips:** None. **N. Davis:** None. **K. Montgomery:** None. **K. Maynard:** None. **K. Martinowich:** None. **J.J. Day:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NARSAD/BBRF Young Investigator Grant
NIH grant MH114990
NIH grant DA039650
NIH grant DA048348

Title: Multiomic profiling of the rat nucleus accumbens reveals cell-type specific chromatin remodeling and transcriptional alterations after cocaine experience

Authors: *J. J. TUSCHER[1], R. A. PHILLIPS, III[1], E. WAN[1], M. E. ZIPPERLY[1], C. G. DUKE[1], L. IANOV[1], S. L. FULTON[2], A. LEPACK[2], I. S. MAZE[2], J. J. DAY[1];
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Abstract: Cocaine use elevates dopamine levels in the nucleus accumbens (NAc) and alters transcriptional programs believed to promote long-lasting synaptic and behavioral adaptations. However, even with well-studied drugs of abuse such as cocaine, transcriptional responses remain poorly understood. This is in part due to the cellular heterogeneity of the NAc, which contains multiple neuronal and non-neuronal cell types, and the complex actions induced by drugs of abuse via multiple neurotransmitter systems. Here, we performed single-nucleus RNA-sequencing (snRNA-seq) on nuclei isolated from male and female rats after acute (15,631 nuclei) or repeated (23,557 nuclei) cocaine exposure to generate a molecular atlas of the NAc. This approach revealed 16 transcriptionally distinct cell subtypes in the NAc, and identified population-specific transcriptional responses to initial cocaine experience, including a core set of activity-dependent immediate early genes. Next, to examine long-lasting chromatin and transcriptional alterations induced by volitional drug use, we performed multiomic profiling with snRNA-seq and single-nucleus Assay for Transposase Accessible Chromatin (snATAC-seq) on 39,325 nuclei from the rat NAc after a 30 day withdrawal period from extended access cocaine self-administration. Together, these datasets reveal a cell-type specific immediate early gene signature engaged by initial drug use, and identify long-lasting transcriptional and chromatin alterations maintained 30 days after drug taking. These results provide key insights into how cellular diversity contributes to chromatin remodeling and transcriptional alterations to distinct types of cocaine experience, and suggest the importance of cell-type specific gene targets for the treatment of substance use disorders.

Disclosures: J.J. Tuscher: None. **R.A. Phillips:** None. **E. Wan:** None. **M.E. Zipperly:** None. **C.G. Duke:** None. **L. Ianov:** None. **S.L. Fulton:** None. **A. Lepack:** None. **I.S. Maze:** None. **J.J. Day:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant MH114990
NIH Grant DA039650
NIH Grant DA048348
UAB Pittman Scholar Program
UAB AMC21 Scholars Program
Brain and Behavior Research Foundation Young Investigator Grant

Title: An atlas of transcriptionally defined cell types in the rat ventral tegmental area

Authors: *R. PHILLIPS[1], J. J. TUSCHER[1], D. FITZGERALD[1], S. BLACK[1], L. IANOV[1], J. J. DAY[2];
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Abstract: The ventral tegmental area (VTA) is a complex brain region that is essential for reward function, but is also implicated in neuropsychiatric diseases including substance abuse. While decades of research on VTA function have focused on the role of dopaminergic neurons, recent evidence has identified critical roles for VTA GABAergic and glutamatergic neurons in reward processes as well. Interestingly, molecular characterization has revealed that subsets of these neurons express genes involved in the transport, synthesis, and vesicular packaging of multiple neurotransmitters, providing evidence for co-release neurons. However, these studies have largely relied on low-throughput methods, and the molecular architecture of the VTA has not been comprehensively examined. Here, we performed single nucleus RNA-sequencing (snRNA-seq) on 21,600 VTA cells from male and female adult Sprague-Dawley rats to generate a transcriptional atlas of the rat VTA. We identified 16 transcriptionally distinct cell types within the VTA, including 7 neuronal populations. Further subclustering revealed several VTA neuronal populations expressing markers for more than one neurotransmitter system, with one cluster exhibiting high expression levels of genes involved in the synthesis and transport of GABA, glutamate, and dopamine. Finally, snRNA-seq enabled the de novo identification of thousands of marker genes for each transcriptionally distinct population, revealing cluster-specific enrichment of gene sets implicated in neuropsychiatric and neurodevelopmental disorders, as well as specific phenotypes associated with alcohol and tobacco use. Together, these results highlight the heterogeneity of cellular populations in the VTA and identify novel markers and disease-linked genes enriched in distinct neuronal subtypes.

Disclosures: R. Phillips: None. **J.J. Tuscher:** None. **D. Fitzgerald:** None. **S. Black:** None. **L. Ianov:** None. **J.J. Day:** None.

Poster

P753. Cocaine: Neuronal Subtypes and Cell Signaling Factors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P753.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01MH123523
BBRF NARSAD Young Investigator Grant #22811

Title: Epigenetic effects of sex and early-life stress on cocaine addiction vulnerability

Authors: D. ROCKS[1], I. JARIC[1], J. M. GREALLY[2], M. SUZUKI[2], ***M. KUNDAKOVIC**[1]; [1]Dept. of Biol. Sci., Fordham Univ., Bronx, NY; [2]Ctr. for Epigenomics, Dept. of Genet., Albert Einstein Col. of Med., Bronx, NY

Abstract: Early-life traumas, such as neglect and abuse, significantly increase the risk for drug abuse and addiction, including cocaine use disorder, in later life. In addition, there are sex differences in cocaine addiction, with females progressing more rapidly into substance use disorder than males, which is at least in part due to sex hormone effects. However, the interaction between sex and stress and, in particular, the biological mechanisms underlying stress- and sex hormone-induced addiction vulnerability remain poorly understood. To study this, we used an early-life stress (maternal separation) paradigm in mice, which included 3-hour daily separations of pups from their mothers from postnatal day P1-14. We then performed the cocaine-induced conditioned place preference (CPP) from P54-60, with the low (2.5 mg/kg) or high (10 mg/kg) cocaine dose, and RNA-seq analysis of the key brain reward area nucleus accumbens (NAc) at P54. We show that early-life stress increases cocaine preference in a sex-dependent manner. Early-life stress-exposed male mice showed cocaine preference after receiving the suboptimal, low dose, not sufficient to induce CPP in controls. In females, both low and high cocaine doses had stronger effects in maternally-separated mice than in controls. Cocaine CPP also varied with the estrous cycle in females, with early-life stress skewing this behavior toward higher preference. Interestingly, we also found female-biased gene expression changes in the NAc in response to early-life stress, involving synaptic transmission-, brain disorder-, and estrogen receptor signaling-relevant genes and pathways. To test whether epigenetic mechanisms are involved in sex-specific and estrous cycle-dependent cocaine responses, at P54, we performed chromatin accessibility (ATAC-seq) analysis of neuronal nuclei from the NAc of proestrus (high-estrogenic) females, diestrus (low-estrogenic) females and males, following 1-hour and 4-hour cocaine (10 mg/kg) treatments. We found sex-specific chromatin changes in the NAc; ~1,000 gene regions in NAc neurons showed differential chromatin accessibility following cocaine exposure in both male and proestrus female mice, with a very little overlap between sexes. Chromatin differences were found nearby genes involved in neuronal function, synaptic plasticity, and the brain reward pathway, implicating different genes and pathways in cocaine-induced plasticity in males and females. Overall, our study increases an understanding of the role of stress and sex hormones in addiction vulnerability, and reveals candidate epigenetic mechanisms underlying sex differences in drug abuse and addiction.

Disclosures: D. Rocks: None. **I. Jaric:** None. **J.M. Greally:** None. **M. Suzuki:** None. **M. Kundakovic:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.01

Topic: F.02. Behavioral Neuroendocrinology

Support: F32MH118782
R01MH107886
2R15GM118304-02
Alzheimer's Association SAGA-17-419092

Title: Estradiol interacts with extracellular matrix metalloproteinase-9 (MMP-9) to enhance hippocampal memory consolidation

Authors: *K. S. GROSS, C. M. LINCOLN, M. M. ANDERSON, G. E. GEIGER, K. M. FRICK;
Psychology, Univ. of Wisconsin, Milwaukee, WI

Abstract: The potent estrogen 17β-estradiol (E_2) is a powerful modulator of hippocampal plasticity and memory. Research on molecular mechanisms of hippocampal E_2 signaling has uncovered multiple intracellular pathways that contribute to these effects, but few have questioned the role that extracellular signaling processes may play in E_2-induced plasticity. The extracellular matrix (ECM) is increasingly recognized as a critical synaptic component, and ECM modifying enzymes like matrix metalloproteinase-9 (MMP-9) are required for hippocampal learning and memory. Little is established about the extent to which E_2 regulates MMP-9 in the hippocampus, and the influence this interaction may have on hippocampal memory is unknown. Therefore, we sought to determine whether E_2 increases MMP-9 activity in the hippocampus to promote memory consolidation on object placement and recognition tasks. Female C57BL/6 mice were ovariectomized and cannulated in the dorsal hippocampus (DH) and dorsal third ventricle (ICV). Immediately following object training, mice were infused with vehicle or a non-memory impairing dose of an MMP-9 inhibitor into the DH and vehicle or E_2 into the ICV. Object placement or object recognition memory was then tested 24 or 48 hours later, respectively. We found that MMP-9 inhibition blocked the memory enhancing effects of E_2, suggesting that ECM modification may be a critical factor in estrogenic regulation of memory. Further experiments found that E_2 rapidly increases hippocampal MMP-9 activity without influencing its protein expression. Continuing work is examining mechanisms and outcomes of E_2-induced MMP-9 activation.

Disclosures: K.S. Gross: None. **C.M. Lincoln:** None. **M.M. Anderson:** None. **G.E. Geiger:** None. **K.M. Frick:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01DA029613

Title: Chronic high-dose testosterone does not decrease myelination of corticostriatal tracts in rat brain

Authors: *A. DONOVAN[1], R. I. WOOD[2];
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Abstract: INTRO- Anabolic androgenic steroids (AAS) are controlled substances. Despite this, AAS are used by professionals and amateurs to improve performance and increase muscularity. AAS are associated with risky behavior, aggression, and reduced connectivity between cognitive and limbic networks in the brain. Increased risk taking is correlated with lowered connectivity of the prefrontal cortex (PFC) and the nucleus accumbens (Acb). Both neurons and glia have been shown to be sensitive to AAS, even though myelinating oligodendrocytes do not express androgen receptor (AR). Testosterone increases remyelination after demyelinating injury in an AR-dependent fashion, but can exaggerate neuronal apoptotic effects when applied at supraphysiologic doses. This suggests androgens moderate myelination, potentially via axo-myelinic signaling. Accordingly, we hypothesized that myelination of the medial corticostriatal tract connecting PFC to Acb would be sensitive to AAS. If so, AAS may impair communication through the medial corticostriatal tract, leading to decreased executive function, and the adoption of high-risk/high-reward behaviors.
METHODS- Male Long-Evans rats received high-dose testosterone (7.5 mg/kg daily, 5d/wk) or vehicle (n=8 each) for 7 weeks. They were sacrificed and brains sectioned coronally at 40 um. We evaluated myelin in the medial corticostriatal tract and forceps minor of the corpus callosum. As positive and negative controls, we also examined the stria terminalis, a tract connecting steroid-sensitive regions in ventral forebrain, and the mid-body of the corpus callosum, a tract connecting steroid-insensitive cortical regions. Myelination was compared using immunohistochemistry for Myelin Basic Protein (MBP), and Oligodendrocyte 2+ (Olig2+) as an oligodendrocyte-specific nuclear marker. Photomicrographs were analyzed using NIH ImageJ.
RESULTS- MBP measurements of fiber tract area showed no effect of testosterone. Olig2+ measurements of normalized stained area, mean gray value, oligodendrocyte cell count, and integrated optical density showed no effect of testosterone. Across all measurements there was a

significant effect of tract on MBP and Olig2+ staining, even after adjusting for differences in tract area.
CONCLUSIONS- AAS increase risk-taking and aggressive behavior. Studies examining the neurobiological basis of these changes have previously focused on neuronal effects of AAS. Our study explored myelination as a new potential mechanism. Although we found no effect of testosterone on myelination at the light microscopic level, examination at the ultrastructural level has potential to reveal further effects.

Disclosures: A. Donovan: None. **R.I. Wood:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.03

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01MH107886

Title: Effects of estradiol treatment on ubiquitin-proteasome activity in the dorsal hippocampus of male and female mice

Authors: *S. B. BEAMISH[1], K. S. GROSS[1], R. K. KUEHN[1], T. J. JAROME[2], K. M. FRICK[1]; [1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Virginia Tech., Blacksburg, VA

Abstract: Sex steroid hormones like 17β-estradiol (E_2) enhance hippocampal function and long-term memory formation in both sexes, however, the molecular mechanisms through which E_2 exerts its effects remain unclear. One overlooked mechanism that may play a key role in the memory-enhancing effects of E_2 in both sexes is protein degradation mediated by the ubiquitin-proteasome system (UPS). In this system, proteins are tagged with multiple ubiquitin modifications at lysine 48 (K48 polyubiquitination) to become substrates for degradation by the 26S proteasome complex. The 26S proteasome is a multi-subunit structure that is activated by phosphorylation of its regulatory subunits following synaptic activity. Preliminary data from our lab demonstrate sex differences in hippocampal proteasome activation following object training, such that males, but not females, have increased phosphorylation of the proteasomal regulatory subunit RPT6 relative to male homecage controls. However, the extent to which these learning-induced sex differences are regulated by sex-steroid hormones such as E_2 is unknown. The present study examined whether dorsal hippocampal (DH) infusion of E_2 alters UPS activity in male and ovariectomized female mice. Adult male and female C57BL/6 mice were implanted with bilateral guide cannulae into the DH and females were then bilaterally ovariectomized. Following surgical recovery, mice received DH infusion of either E_2 or vehicle, and DH tissue was collected 4 and 6 h later for Western blot analysis of K48 polyubiquitinated proteins. Our

findings indicate that DH infusion of E_2 increases degradation-specific K48 polyubiquitination in both sexes, however, E_2-treated males have increased K48 tagging at 6 h, whereas E_2-treated females have increased K48 tagging at both 4 and 6 h. These findings suggest that E_2 treatment alone stimulates ubiquitin tagging to a greater degree in females than in males. Current work is examining whether E_2-induced increases in K48 polyubiquitination concomitantly increase proteasome activity and whether proteasome activity is required for successful spatial and object recognition memory consolidation in gonadectomized male and female mice. This work ultimately aims to reveal a potential new mechanism through which E_2 regulates hippocampal memory formation in male and female mice.

Disclosures: S.B. Beamish: None. **K.S. Gross:** None. **R.K. Kuehn:** None. **T.J. Jarome:** None. **K.M. Frick:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.04

Topic: F.02. Behavioral Neuroendocrinology

Support: Congressionally Directed Medical Research Program Autism Idea Award AR110109
Cardiovascular Research and Training in Texas T32
Summer Physiology Research Program R25
The Morrison Trust

Title: Chronic metformin treatment effects on steroid hormones and behaviors relevant to autism

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Abstract: Despite increases in diagnoses of autism spectrum disorder, there still remains no comprehensive treatment to combat the debilitating restrictive-repetitive and inhibited social behavioral symptoms. Often adolescents have multiple prescriptions, with metformin commonly co-administered to offset weight gain caused from antipsychotics. Metformin has been reported to have other properties, such as androgen suppression that may counter increased steroid levels found in many children with autism. For this project, we focused on testing the hormonal suppression effects caused by metformin as a means to ameliorate the behavioral symptoms of autism using a mouse model. BTBR T+Itpr3tf/J (BTBR) mice were used because this strain is a popular behavioral autism model with high testosterone, corticosterone, and progesterone levels, and low serotonin (5-HT) transporter (SERT) density as compared to C57BL/6J (C57) mice,

which were used as a strain control. Acute IP injections with metformin in BTBR enhanced social behavior in the three-chamber social interaction and social novelty test. Therefore, metformin was administered chronically over six weeks via drinking water to see if the effect remained consistent. Mice were tested in several behaviors including three-chamber tests for social interaction and social novelty, marble burying for restrictive repetitive behavior, tube test for social dominance, and water t maze for cognitive flexibility. Once behavioral testing ended, serum was collected to measure testosterone, corticosterone, and progesterone, in addition to fasting blood glucose. Overall, metformin exposure did not prove effective at increasing BTBR social behavior in the three-chamber test, but it did increase dominance in the tube test for both BTBR and C57 mouse models. Marble burying by mice over 30 min did not differ by strain, sex or metformin treatment. Cognitive flexibility in the water t maze, measured by number of errors, percent of error free trials, and time to swim to the platform showed negative impacts for both number of errors and time swimming on C57 mice after exposure to chronic metformin. Physiological measures showed no changes in steroid hormones, and increased blood glucose after exposure to chronic metformin in both BTBR and C57 mouse models. Overall, our findings indicate chronic use of metformin may increase social dominance but impair cognitive flexibility via mechanisms independent of blood glucose or steroid hormones.

Disclosures: S. Greene: None. **B. Bordas:** None. **G. Alcala:** None. **P. Jimenez:** None. **Y.R. Sanchez:** None. **M.K. Penta:** None. **L.C. Daws:** None. **G.G. Gould:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.05

Topic: F.02. Behavioral Neuroendocrinology

Support: Sex and Gender in Alzheimer's SAGA-17-419092

Title: Effects of dorsal hippocampal 17b-estradiol infusion and APOE genotype on dendritic spine densities in the hippocampus and prefrontal cortex in a mouse model of Alzheimer’s Disease

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Abstract: Rationale: The menopausal loss of circulating hormones is associated with heightened risk for the development of Alzheimer’s Disease (AD) in women relative to men. Additionally, women carriers of the *APOE4* allele, the greatest genetic risk for developing AD, are at higher risk of developing AD relative to women of any other *APOE* genotype. Thus, there appears to be a strong link between hormonal status and *APOE* genotype which could mediate

cognitive decline in AD and provide a potential therapeutic target to improve outcomes in women with AD. Our lab and others have demonstrated that infusion of 17β-estradiol (E2), the potent estrogen, into the dorsal hippocampus (DH) improves spatial and object recognition memories in ovariectomized (OVX) wildtype mice, effects associated with proliferation of dendritic spines in the DH and medial prefrontal cortex (mPFC). However, whether E2 affects dendritic spine densities similarly in a mouse model of Alzheimer's Disease has yet to be determined. **Objective:** The goal of this study was to determine the effects of DH-infused E2 on basal and apical spines in the DH and mPFC. **Methods:** 6-month-old female EFAD mice ($5xFAD^{+/-}/APOE^{+/+}$), including E3FAD, E4FAD, and E3/4FAD were ovariectomized. Following behavioral testing, mice were infused bilaterally into the DH with vehicle or E2. Mice were euthanized 2 hours later, and their brains were collected for Golgi staining. Secondary and tertiary apical spines and secondary basal spines were counted in the CA1 subfield of the dorsal hippocampus and layer II/III of the prelimbic mPFC. Spines were classified as stubby, thin, or mushroom, based upon their morphology. **Results:** Preliminary results show that direct DH infusion of E2 increased CA1 apical, but not basal, spines in E3FAD and E3/4FAD mice 2 hours later. E4FAD mice had reduced basal and apical spine densities in CA1 compared to E3FAD and E3/4FAD mice, regardless of E2 treatment. Finally, our preliminary results suggest no effect of genotype or DH E2 infusion on basal or apical dendritic spine densities in the mPFC. **Conclusions:** E2 infusion into the DH of E3FAD and E3/4FAD, but not E4FAD, OVX mice facilitates dendritic spine growth in the DH, but this effect does not extend to the mPFC, a downstream target of DH neurons. Given that DH infusion of E2 increases spine densities in the mPFC of young, OVX wildtype mice, this suggests disrupted circuitry in EFAD mice that cannot be overcome by E2's actions in the DH. Therefore, although E2 may be a promising treatment for memory deficits in AD, further work is needed to elucidate the mechanisms by which it does so both at the local and circuit levels.

Disclosures: A.W. Fleischer: None. **L.R. Taxier:** None. **K.M. Frick:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.06

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01AG041374

Title: Rapid synthesis of estrogen is required for hippocampal long-term plasticity in absence of ovarian hormones in female mice.

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Abstract: Menopause is characterized by cessation of ovarian function, leading to a decrease in the level of circulating estrogens which support neuronal functions. Although still debated in humans, estrogens, mainly 17-β-estradiol (E2), improve memory related behavioral tasks in rodents. Estrogens enhance connectivity and neuronal plasticity, which underpin memory formation, consolidation, and maintenance. For instance, E2 enhances excitatory neurotransmission through an NMDA receptor-dependent mechanism as well as an increase in pre-synaptic release of glutamate. It also facilitates and enhances long-term potentiation (LTP) in the hippocampus. In addition to ovarian synthesis, estrogens are locally produced in the brain of both sexes. Importantly, the enzyme p450 aromatase (ARO) which converts testosterone into E2 has been found in several brain areas, including the hippocampus. This local synthesis of E2 can support estrogen-dependent transcription and blocking it with ARO inhibitors like letrozole (Let) impairs hippocampus-dependent memory as well as LTP. Although existing evidence points to a critical role of local estrogen synthesis in hippocampal memory formation, little is known on the regulation of this process, particularly after the loss of ovarian functions.
In this study we tested the hypothesis that rapid, local and on demand synthesis of E2 is necessary for LTP after removal of ovarian hormones. To address this question, we performed field recordings of evoked post synaptic potentials on hippocampal slices of ovariectomized mice. We blocked ARO activity by applying Let for 30 minutes and induced LTP at the CA3 to CA1 synapses using a theta burst protocol. We found that in the absence of ovarian estrogen, blocking ARO attenuated the development of LTP. We also verified that the ARO inhibition did not affect the response to the stimulation or the presynaptic release of neurotransmitters. These findings support a role for locally synthesized neuroestrogens as regulators of hippocampal synaptic plasticity after cessation of ovarian functions. Better understanding of the mechanism regulating neuroestrogen production in the brain could help therapy development against cognitive aging.

Disclosures: M. Maroteaux: None. **J.M. Daniel:** None. **L.A. Schrader:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.07

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01AG041374

Title: Memory Impairment in Female Mice Lacking Membrane Estrogen Receptor Alpha

Authors: *A. F. DELARGE[1,2], M. J. STANLEY[3], J. M. DANIEL[1,2,3];
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Abstract: Previous work from our lab has shown the capacity of estradiol, the primary estrogen produced by the ovary, to improve spatial memory in rodents. These memory enhancing effects are mediated in part by estrogen receptor alpha (ERα) which can be localized to the nucleus and cellular membrane. ERα located in the nucleus utilize the classical genomic signaling pathway which leads to transcriptional changes that occur over hours to days. In contrast, membrane ERα receptors utilize a rapid signaling pathway with effects occurring in seconds to minutes. In order to examine the effects of ERα cellular location on spatial memory, female mice containing only nuclear ERα (NOER) and wildtype (WT) female mice underwent acquisition training on an eight-arm radial maze. Delay-dependent effects on memory were tested by inserting a delay, which ranged from 30 minutes to 5 hours, between the 4th and 5th arm choices. Performance was assessed by number of proactive errors made, defined as reentry into an arm previously visited in the current trial, and number of retroactive errors made, defined as reentry into an arm previously visited prior to the delay period. Results revealed that NOER mice made significantly more proactive errors across trials than WT mice, regardless of delay. Conversely, the effect on retroactive errors was delay-dependent, and NOER mice made significantly more errors than WT mice after a 5-hour delay. Despite significant differences in spatial memory, NOER and WT mice displayed similar anxiety-related behavior and locomotor activity as measured by the elevated plus maze and open field test, respectively. These results suggest that membrane ERα plays an important role in short term working memory as well as delay-dependent memory as indicated by increases in both proactive and retroactive errors made by NOER mice. Although, significant increases in retroactive errors were not seen until the longest delay period when memory load was highest. In addition, behavioral differences did not extend to anxiety-related behavior or locomotor activity, suggesting that the effects of lacking membrane ERα are specific to memory. However, because NOER mice have superphysiological estradiol levels due to an impaired negative feedback loop, further experiments are needed to confirm that memory impairment in NOER mice is a result of the absence of membrane ERα and not increased levels of circulating estradiol. Nevertheless, this study determined that female mice lacking the rapid actions of membrane ERα exhibit spatial memory impairment in comparison to WT controls.

Disclosures: A.F. DeLarge: None. **M.J. Stanley:** None. **J.M. Daniel:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.08

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH RF1AG041374

Title: History of previous midlife estradiol treatment permanently alters brain estrogen receptor signaling by maintaining interactions between insulin-like growth factor-1 signaling and neuroestrogen synthesis to enhance cognitive aging in a rat model of menopause

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Abstract: The loss of ovarian hormones during menopause coincides with cognitive decline and increased risk of age-related dementias. Due to serious health risks associated with prolonged estrogen exposure, current health guidelines recommend using postmenopausal estrogen treatment for as short a time as possible. Work from our lab in a rodent model of menopause has demonstrated long-lasting benefits of short-term estradiol treatment on hippocampal function and memory through sustained activation of estrogen receptor (ER) α that persists long after estradiol treatment is terminated. A history of previous midlife estradiol treatment enhances performance on the radial-arm maze and increases levels of hippocampal ERα in aging ovariectomized rodents, an effect that is dependent on insulin-like growth factor-1 receptor (IGF-1R) and results in sustained ER-dependent transcriptional activity. The results of the current experiments demonstrate a mechanism through which the activation of hippocampal ERα can enhance memory following long periods of ovarian hormone deprivation. First, we showed the necessary role of brain-derived neuroestrogens in IGF-1-mediated activation of ERα via the MAPK pathway and its subsequent enhancements in memory in aging ovariectomized rats. Then we tested the interactive relationship between neuroestrogens and IGF-1 receptors in activating hippocampal ERα using two models of menopause in rats: one in which long-term ovariectomized aging females received no estradiol treatment in midlife and one in which long-term ovariectomized aging females received midlife estradiol treatment that was terminated after 40 days. In the long-term ovariectomized rats that did not receive previous midlife estradiol treatment, IGF-1R and neuroestrogens were no longer able to interact to activate ERα and impact memory. However, a history of previous midlife estradiol treatment reversed this effect and facilitated maintenance of the interactive relationship between IGF-1R and neuroestrogens, resulting in sustained increases in activation of ERα and enhanced memory. Together, these results provide a potential model for combatting postmenopausal cognitive decline in which short-term estradiol treatment following the loss of ovarian hormones sustains hippocampal function and memory beyond the period of estradiol treatment by permanently altering the dynamic relationship between IGF-1R signaling and neuroestrogen synthesis, allowing for long-term activation of hippocampal ERα.

Disclosures: N.E. Baumgartner: None. **S.M. McQuillen:** None. **S.M. Miller:** None. **J.M. Daniel:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.09

Topic: F.02. Behavioral Neuroendocrinology

Support: Oscar Stern Award/Eisenberg Family Depression Center University of Michigan to NCT
BP-ENDURE summer funds to IJPO

Title: A mouse model of oral contraceptive exposure: effects on depression-like behavior

Authors: *K. M. SCHUH[1], I. J. PAT-OSAGIE[2], T. T. DAVIS[1], J. AHMED[1], N. C. TRONSON[1];
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Abstract: Hormonal contraceptives (HCs), including combined oral contraceptives, are an important part of women's healthcare and confer massive economic benefits to women worldwide. 85% of women in the United States use this form of contraceptive at some point in their lifetime with 14% currently using oral contraceptives and 11% currently using long-acting reversible contraceptives. Despite the massive number of women that use them, HCs affect mood, behavior, and the brain in ways that are not fully understood. Anecdotally, some women report increased depression and anxiety, a finding that is corroborated by some studies, while others have found that HCs are protective against mood disorders. Studying whether and how HCs contribute to risk and resilience of depression, anxiety, and other stress-related disorders is extremely difficult with human participants due to multiple formulations of HCs, and individual differences in durations of HC use, genetic predispositions to psychiatric disorders, and prior/current stress exposures. The current project aimed to develop and validate a mouse model of HC-like exposure by examining the effects of two different combinations of hormones (0.075 μg/mL ethinyl estradiol and either 3 μg/mL levonorgestrel or 15 μg/mL drospirenone in 0.25mL of 10% sucrose, daily at 5pm) on vulnerability to depressive-like and anxiety-like behaviors in mice after subchronic variable stress. Estrous cycles were tracked using both visual and vaginal cytology methods. We found that HCs alone did not affect locomotor activity, behavioral despair, or memory in a context fear conditioning task. However, HC exposure did decrease both sucrose drinking and sucrose preference, suggesting that HC exposure may specifically affect motivational circuits and motivation- or anhedonic-related components of depression. Overall, we demonstrate that this mouse model of hormonal contraceptive-like exposure is a valid and tractable method for identifying effects of oral contraceptives on the brain, including how HCs affect hypothalamic-pituitary-adrenal axis regulation and thereby influence risk or resilience to depression.

Disclosures: K.M. Schuh: None. **I.J. Pat-Osagie:** None. **T.T. Davis:** None. **J. Ahmed:** None. **N.C. Tronson:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.10

Topic: F.02. Behavioral Neuroendocrinology

Support: Oscar Stern Award/Eisenberg Family Depression Center University of Michigan to NCT
BP-ENDURE summer funds to IJPO

Title: Regulation of the acute stress response by oral contraceptive hormones

Authors: I. J. PAT-OSAGIE[1], K. M. SCHUH[2], T. T. DAVIS[2], J. AHMED[2], *N. C. TRONSON[2];
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Abstract: "The Pill" and other hormonal contraceptives (HCs) are a central part of women's healthcare. They provide control over reproductive timing, and additional health benefits to many individuals. For some people, HCs trigger depression and other negative affective states; yet for others, HCs exert protective effects against depression. In this project, we aim to understand how interaction between HC hormones and the HPA axis contributes to vulnerability or resilience to negative affective states. Specifically, we aimed to determine how different progestins commonly used in HCs regulate the stress response. We used a mouse model of HC hormone exposure recently developed in our lab, ELISA assays to assess corticosterone concentration, and cFos immunohistochemistry to identify stress-induced activation of brain regions and networks. We examined both C57Bl/6N and the more stress-responsive Balb/c mice to understand how individual differences in stress responsivity might contribute to changes in HC-induced risk for depression. Young adult female mice were given either ethinyl estradiol (EE; 0.075 μg/mL) and levonorgestrel (LNG; 3 μg/mL), a progestin with androgenic properties; or EE and drospirenone (DRSP; 15 μg/mL), a progestin with anti-androgenic properties, in a 10% sucrose suspension (0.25 mL) daily at 5pm. Based on data from women HC users, we hypothesized that HC hormone exposure would cause suppression of the corticosterone response to an acute stressor. We observed that mice exposed to EE+LNG showed significant blunting of the stress response as measured by serum corticosterone concentration. Surprisingly, mice treated with EE+DRSP did not show this effect. These data demonstrate that different progestins differentially modulate the HPA axis, thus progestin androgenicity may be a central factor in altered risk or resilience to depression for women on HCs. Future work will further establish whether the effects of different progestins on stress response are mediated via androgenic receptors, or via mineralocorticoid and glucocorticoid receptors. This work lays the foundation

for guiding the choice of HC formulation to increase individual women's resilience to negative affective states including depression.

Disclosures: I.J. Pat-Osagie: None. **K.M. Schuh:** None. **T.T. Davis:** None. **J. Ahmed:** None. **N.C. Tronson:** None.

Poster

P855. Hormones and Cognition: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P855.11

Topic: F.03. Neuroendocrine Processes

Support: Harvey L. Karp Discovery Award (CT)
Brain and Behavior Research Foundation (EGJ)
National Institutes of Health/National Institute on Drug Abuse (NIH/NIDA) F32DA038927(DF)
National Institutes of Health/National Institute on Drug Abuse (NIH/NIDA) R01DA034685 (MD)

Title: Differences in striatal dopamine synthesis and task switching performance between hormonal contraceptive users and non-users

Authors: *C. M. TAYLOR[1], D. J. FURMAN[3], A. S. BERRY[5], R. L. WHITE[4], W. J. JAGUST[6], M. DESPOSITO[7], E. G. JACOBS[2];
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Abstract: Accumulating evidence suggests that sex steroid hormones regulate dopamine (DA) neurotransmission. In rodents, 17ß-estradiol and progesterone modulate DA synthesis and release, and impact the basal firing rate of DA-ergic neurons. In nonhuman primates, DA-D2 receptor availability varies across the menstrual cycle. Further, sex hormone receptors have been localized to regions that receive major projections from midbrain DA neurons, including prefrontal cortex, dorsal striatum and the nucleus accumbens. However, much less is known about hormonal regulation of the dopamine system in the human brain. Here, we begin to address this gap by comparing dopaminergic function between female hormonal contraceptive users and non-users in a multimodal molecular PET imaging study. Pre- and postsynaptic DA signaling was assessed along three domains: dopamine synthesis capacity using the PET radioligand 6-[^{18}F]fluoro-l-m-tyrosine ([^{18}F]FMT), baseline D2/3 receptor-binding potential using [^{11}C]raclopride, and dopamine release using methylphenidate-paired [^{11}C]raclopride PET.

Participants consisted of 36 healthy females (age 18-28 years old, n=15 hormonal contraceptive users, HC; n=21 naturally cycling females, NC). Cognitive flexibility, a behavioral measure previously shown to be sensitive to DA synthesis, was also assessed. Results indicate that hormonal contraceptive users exhibit greater dopamine synthesis capacity (greater ROI-based, partial volume corrected [^{18}F]FMT K_i values) than naturally cycling females in dorsal caudate (NC_{avg}= 0.0256, HC_{avg} = 0.0295; $t(34) = -4.29$, $p = .0001$, $d = -1.49$) as well as greater cognitive flexibility (indexed as a lower behavioral switch cost on a task switching paradigm) ($t(28.99) = -2.0763$, $p = 0.0468$). We observed no group differences in D2/3 receptor binding or dopamine release. Together, this study provides a unique opportunity to examine differences in basal dopamine receptor occupancy, stimulated dopamine release, and dopamine synthesis capacity in a single cohort, based on women's hormonal contraceptive status. Hormonal contraception (in the form of pill, shot, implant, ring or IUD) is used by ~300 million women worldwide, yet few studies have examined how chronic hormone manipulations impact basic properties of the dopamine system. Findings from this study begin to address this critical gap in women's health.

Disclosures: C.M. Taylor: None. **D.J. Furman:** None. **A.S. Berry:** None. **R.L. White:** None. **W.J. Jagust:** None. **M. Desposito:** None. **E.G. Jacobs:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.01

Topic: H.11. Language

Title: On characterizing the narrative speech N400 using semantically-tuned language models

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Abstract: The human brain understands speech heard at rates of around 120–200 words per minute. A well-known signature of this process is the N400 electrophysiological component – a prominent centroparietal negativity that occurs 200-600ms after word onset. Although the N400 has been the subject of thousands of research studies, what it represents is incompletely understood. This is especially so for continuous natural speech. One possibility is that the N400 is the product of a neural predictive processing mechanism(s) that: (1) anticipates linguistic properties of upcoming words, and (2) generates an error signal when predictions are wrong reflecting the degree of inaccuracy (or surprise). Furthermore, error signals may be generated in parallel at multiple levels of a linguistic processing hierarchy, thus there may be semantic, syntactic and discourse-level error components contributing to the N400 signal. Computational language-models that are trained to predict properties of upcoming words (much

the same as the brain mechanism underpinning the N400 is thought to do) present new opportunities for interrogating neural responses to narrative speech. We here present pilot work seeking to better characterize neural processing at the semantic level using a tuned language model, as a precursor for future more comprehensive tests. Specifically, we took a "baseline" language model pretrained to predict the identity of upcoming words (XL-Net) and fine-tuned that on a semantic-role labelling task to produce high quality semantic representations attuned to "who did what to whom". We hypothesized that this semantic tuning would improve our ability to predict EEG signal in the N400 range.
We tested this hypothesis on a pre-existing EEG dataset recorded as 19 participants listened an audiobook. We computed a semantically-tuned N400 predictor by computing the correlation-dissimilarity between the final layer of network activations for the current word and the word preceding it. We repeated this for the baseline model. Dissimilarity values were placed at word onset times, lagged to correspond to +200 to +600ms range and aligned to the EEG. Regularized regression was deployed to map the EEG onto the model in a cross-validation framework. The regression mapping was evaluated by predicting held out EEG data. Mean prediction accuracies (across channels) were significantly greater for the semantically tuned model than the baseline (signed rank=190,p=1e-4,n=19). These results suggest that semantic-tuning led to a better characterization of semantic processing in the brain and provide a promising foundation for future research based on computational language modeling.

Disclosures: J. Dou: None. **A.J. Anderson:** None. **A. White:** None. **E.C. Lalor:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.02

Topic: H.11. Language

Support: NIH Grant P50-MH109429
Simons Center for the Social Brain Targeted Project: Predictive Processes

Title: Neurophysiological timescales in dynamic Bayesian inference of syllable segmentation and speech speed

Authors: *B. PITTMAN-POLLETTA[1], J. CANNON[2], L. DILLEY[3];
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Abstract: Evidence increasingly suggests that both the timing and content of speech contribute to speech segmentation and understanding, and that neuronal rhythms play key roles in these computations. Yet the computational principles by which timing, content, and top-down

information are combined in speech perception remain poorly understood. To illuminate the potential mechanisms underlying speech segmentation, we construct an explicit computational model of a predictive coding account of speech segmentation, the Syllable Inference (SI) hypothesis. SI was proposed to explain distal rate effects, in which altering the rate of context speech changes the perception of an unaltered target segment, making short words such as the preposition ``a'' appear or disappear. Under the SI hypothesis, candidate speech interpretations, syllable-specific duration estimates, and estimated speech rate are used to infer a predicted duration for each segment that is then compared and updated with acoustic data to yield speech understanding and segmentation. In this work, we propose and implement a Bayesian framework for syllable inference, and apply it to both data-derived artificial stimuli and real speech. In line with recent predictive coding hypotheses, in our framework a forward model and a backward model operate at different timescales, and alternate in time. These alternating prediction and update cycles occur at beta and gamma timescales, respectively, while lexical retrieval operations update the prior on syllable likelihood at a theta timescale. We test this framework using a synthetic language consisting of strings of syllables, with syllabic and phonemic durations taken from the TIMIT corpus, which allows us to derive distal rate effects while abstracting away higher-order linguistic information. Incorporating neurophysiologically realistic timescales at the algorithmic level, our model sheds light on how the timing of human speech perception is constrained by neurophysiology, and on how these timescales shape speech understanding. Our work generates a number of hypotheses, suggesting further experiments and connections to both biophysical computational models of cortical rhythms (at the implementation level) and recent theories about the level of detail in predictive processing (at the algorithmic/computational level).

Disclosures: B. Pittman-Polletta: None. **J. Cannon:** None. **L. Dilley:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.03

Topic: H.11. Language

Title: Development of language-related white matter tracts and their relationships with single word reading ability among healthy children and adolescents

Authors: Y. KIM, P. THOMAS, S. CROWELL, B. LANEY, E. NELSON, W. MATTSON, *K. R. HOSKINSON;
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Abstract: Objectives
As children get older, white-matter fiber tracts often show increased fractional anisotropy (FA), possibly reflecting white matter maturation and pruning. Certain specific tracts, including the arcuate fasciculus (AF), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF), inferior fronto-occipital fasciculus (IFOF), and uncinate fasciculus (UF) are thought to play a role in supporting language processing and reading abilities. In this study, we investigated age-related changes in these fiber bundles among typically developing children and adolescents to examine links among specific white matter pathways and word reading ability.
Methods
Participants included 76 healthy children and adolescents aged 8 to 21 years (27 males, M_{age}=13.86). Participants completed the word reading subtest from the Wide Range Achievement Test, Fifth edition (WRAT-5) and underwent diffusion weighted imaging (DWI) on a Siemens Prisma 3Tesla scanner. Analyses of white matter fiber tracts were completed using Automated Fiber Quantification (AFQ) which yielded quantified FA values of the bilateral AF, ILF, SLF, IFOF, and UF. All statistical analyses were performed using SPSS, and included bivariate correlations and mediation modeling using ordinary least squares path analysis.
Results
As expected, participants scored within the average range on the WRAT-5 Word Reading subtest (*M*=106.55, *SD*=16.34). Bivariate correlations revealed a strong relationship between WRAT-5 raw scores and FA within the left (*r*=.31) and right ILF (*r*=.38; *p*s<.01), though not the other tracts. Age was also positively associated with FA within the right SLF, bilateral ILF, and right IFOF (*r*s=.26 to .32; *p*s<.05) The indirect effect of age on WRAT-5 performance via left ILF was positive but not statistically significant: *b*=.1356, 90%CI [-.0271, .3925]. The indirect effect of age on WRAT performance via right ILF was positive and statistically significant: *b*=.1964, 90%CI [.0347, .3859].
Conclusions
In this sample of healthy children and adolescents, age-linked maturation in the bilateral ILF mirrored improved performance on a measure of word reading, highlighting the role of this fiber pathway on supporting language processing. Moreover, FA in the right ILF accounted for significant variance in word reading, above and beyond that accounted for by age alone. Future analyses will examine more complex language processing tasks to determine the relative contribution of white matter substrates to basic vs. higher-order language.

Disclosures: Y. Kim: None. **P. Thomas:** None. **S. Crowell:** None. **B. Laney:** None. **E. Nelson:** None. **W. Mattson:** None. **K.R. Hoskinson:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.04

Topic: H.11. Language

Support: Schmitt Program on Integrative Neuroscience

Title: Predicting language processing in the speaking brain

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Abstract: Understanding how linguistic meaning is represented in the human brain presents a major scientific challenge. One approach that has emerged from advances in functional neuroimaging, big data, and machine learning, is to computationally model meaning, and use models to predict brain activity. Over the past fifteen years this approach has helped to map out a network of brain regions engaged in representing the meaning of noun, verb, sentence and continuous narrative speech comprehension. To date, one major stone that has remained unturned is the case of speech production. Although we may suppose that the same neural mechanisms process semantics during both speech production and comprehension, direct brain imaging evidence for this is scant. We present initial results of an fMRI study seeking to bridge this gap and chart neural correlates of language processing during natural speech production comparative to comprehension. We tested a pair of participants who spoke ad lib about six general topics (e.g. people, places, events) for a total of 36mins. This was first in a mock scanner to get them accustomed to speaking with minimal head motion. They then took turns to again speak about the topics whilst undergoing fMRI. In a later fMRI visit they listened to the other participant's speech (the higher quality mock-scanner sound recording was used). To enable us to identify neural correlates of high-level language processing, we deployed an artificial neural network language model (Transformer-XL) to represent each word spoken. Transformer-XL is pretrained to predict the identity of upcoming words (which interestingly also enables it to produce language) and layer activations produce word vectors reflecting semantic information structure. To account for spurious covariation between high and low-level features of speech we also modeled articulatory features of spoken phonemes and the acoustic spectrogram. To isolate correlates of linguistic processing in the speaking/listening fMRI data, we mapped each voxel's activation time-series on to a time-lagged representation of the three models using regularized regression in a cross-validation framework. Predictions were evaluated on held out fMRI data. During natural speech production the language model was able to accurately predict fMRI activation in medial parietal, inferior parietal and inferior frontal cortex (all consistent with memory retrieval) but less so in lateral temporal cortex. A similar selection of regions, including lateral temporal cortex, could be predicted in listening. These results provide an exciting glimpse into language processing in the speaking brain and how it relates to listening.

Disclosures: A.J. Anderson: None. **D. Doddell-Feder:** None. **E.C. Lalor:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.05

Topic: H.11. Language

Title: Thinking ahead: spontaneous prediction in context as a keystone of language in humans and machines

Authors: *A. GOLDSTEIN[1], Z. ZADA[1], B. AUBREY[1], E. BUCHNIK[2], S. NASTASE[1], H. GAZULA[1], R. REICHART[4], O. LEVY[5], A. FEDER[2], S. DEVORE[6], C. COSTO[1], L. FANDA[6], A. PRICE[1], M. SCHAIN[2], K. A. NORMAN[1], A. FLINKER[6], A. HASSIDIM[2], M. BRENNER[3], O. ORRIN DEVINSKY[6], U. HASSON[1];
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Abstract: Departing from traditional linguistic models, advances in deep learning have resulted in a new type of predictive (autoregressive) deep language models (DLMs). Using a self-supervised next-word prediction task, these models are trained to generate appropriate linguistic responses in a given context. We provide empirical evidence that the human brain and autoregressive deep language models share three fundamental computational principles as they process natural language: 1) both are engaged in continuous next-word prediction before word-onset; 2) both match their pre-onset predictions to the incoming word to calculate post-onset surprise (i.e., prediction error signals); and 3) both represent words as a function of the previous context. We recorded intracranial recordings from nine patients while free listening to a 30 min podcast to support these three principles. Our findings indicate that: a) the intracranial neural activity before word-onset contains predictive information about forthcoming words, even hundreds of milliseconds before the words are perceived. In addition, autoregressive deep language models can be used to track the content of the brain's pre-onset prediction, even when the predictions are inaccurate; b) the neural activity after word-onset reflects the surprise level and prediction error; c) the autoregressive deep language model's contextual embeddings capture the neural representation of context-specific word meaning better than arbitrary or static semantic embeddings. Together, our findings suggest that autoregressive DLMs provide a novel and biologically feasible computational framework for studying the neural basis of language.

Disclosures: A. Goldstein: None. **Z. Zada:** None. **B. Aubrey:** None. **E. Buchnik:** None. **S. Nastase:** None. **H. Gazula:** None. **R. reichart:** None. **O. Levy:** None. **A. Feder:** None. **S. Devore:** None. **C. Costo:** None. **L. Fanda:** None. **A. Price:** None. **M. Schain:** None. **K.A. Norman:** None. **A. Flinker:** None. **A. Hassidim:** None. **M. Brenner:** None. **O. Orrin Devinsky:** None. **U. Hasson:** None.

Poster

P885. Neural and Circuit Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P885.06

Topic: H.11. Language

Support: NIH R01 NS109367

Title: Visualizing lexical activation during speech production

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Abstract: Lexical retrieval is central to language, but the spatial and temporal neural codes that subserve it remain elusive. Here we employ direct neural recordings (ECoG) in humans and leverage a machine learning (decoding) approach to elucidate the neural instantiations of two central aspects of lexical retrieval during overt language production: First, the spatiotemporal patterns of neural activity that code for a word's activation. Second, the neural states that correspond to discrete stages of lexical representation (conceptual, phonological, articulatory; Levelt, 1989; Indefrey, 2011). Four patients undergoing neurosurgery for refractory epilepsy repeatedly produced 6 nouns (dog, ninja, etc.) in response to cartoon images while electrical potentials were measured directly from cortex. During a picture naming block, patients saw cartoon characters and responded overtly ("dog"). Subsequently, the same characters engaged in static scenes were shown and patients produced corresponding sentences (e.g. "The dog tickled the ninja"). We were able to predict above chance ($p<0.05$, permutation, accuracy ~=22%) which of the 6 nouns a subject was about to produce in the ~500ms leading to articulation using cross-validated multi-class classifiers. Accuracy increased leading up to production onset and then decreased, suggesting that the classifiers capture a neural process akin to lexical activation rather than signatures of articulatory processing (or early visual features which were removed from analysis). We tested the generalizability of the finding by applying the same trained classifier to nouns produced in sentences, showing above-chance accuracy for the first noun in the sentence.Next, in order to test for discrete neural stages corresponding to lexical processes we employed a temporal generalizability approach: we trained classifiers on each time sample, then tested each of these on held out trials from each time sample (following King & Dehaene, 2014; Gwilliams et al., 2020). If lexical activation involves passing through discrete representational stages, the temporal generalizability pattern should show distinct temporal patches representing neural states. Our results provide direct evidence for 2-4 distinct neural states during lexical retrieval within subjects. These states commenced approximately 600 ms prior to articulation and continued until articulation onset. The neural states likely represent temporally stable patterns supporting conceptual, phonological, and articulatory planning processes. Our results provide an important step towards linking neural spatiotemporal codes to theoretical lexical states.

Disclosures: A. Morgan: None. **W. Doyle:** None. **O. Devinsky:** None. **A. Flinker:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.01

Topic: I.04. Physiological Methods

Support: NIH Grant UF1NS107667
Howard Huges Medical Institute (HHMI)
Laboratory Directed Research and Development Program of Lawrence Berkeley National Laboratory under US DOE Contract No. DE-AC02-05CH11231

Title: Echip: a compact integrated circuit with 512 channels for in vivo electrophysiology

Authors: *J. HERMIZ[1], C. GRACE[2], J. JOSEPH[2], M. L. KARLSSON[5], M. P. KARLSSON[5], K. LEE[6], A. PAPADOPOULOU[2], C. TINDALL[2], L. M. FRANK[7], K. E. BOUCHARD[3], P. DENES[4];
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Abstract: Understanding brain function requires sampling activity from widely distributed neural circuits at high temporal resolution during behavior. While state of the art systems are beginning to enable thousand channel experiments, current systems are limited in their flexibility and dynamic range. First, the lack of high density connectors has driven the development of monolithic silicon probe and hardware solutions that cannot easily be redesigned for many different species or use cases. Second, the analog front-end of the conventional design is space-constrained by the large capacitors needed to AC-couple with a cutoff frequency < 1 Hz. While a DC-coupled circuit would obviate this need for large capacitors and thus be easier to scale, it would have to deal with large DC offsets in the electrode-electrolyte interface. We have created EChip, a novel DC-coupled, high-channel-count integrated circuit for full bandwidth neurophysiology to overcome these challenges. EChip is designed to be bonded directly to a variety of different recording devices, allowing for flexibility and adaptability to many use cases. The 6.9 mm x 8.2 mm chip acquires data from 512 channels at a sampling rate of 30kS/s, and is integrated with a commercially available recording system from SpikeGadgets. Each channel includes amplification, filtering, and a 14-bit digitization. EChip can operate in a purely DC-coupled mode or in a DC feedback mode that keeps the operating point balanced on average. To validate EChip, we performed in vivo recordings in anesthetized rats using a silicon laminar polytrode (Cambridge Neurotech) in the primary auditory cortex while presenting auditory stimuli. We observed prominent stimulus-evoked potentials, where median responses across trials were 10 σ above baseline. Application of spike sorting showed high quality single units and several multi-unit clusters that were modulated by the stimulus. These results demonstrate that

EChip's novel DC feedback circuit can capture neurophysiological signals. While further development and validation are needed, these results pave the way for the planned integration with polymer polytrodes that can record from single units stably for long periods of time. By integrating multiple EChips together, we aim to record from 4096 channels distributed throughout the rat brain over long time scales to advance our understanding of information processing in local and distributed neural circuits.

Disclosures: J. Hermiz: None. **C. Grace:** None. **J. Joseph:** None. **M.L. Karlsson:** A. Employment/Salary (full or part-time):; SpikeGadgets. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SpikeGadgets, NeuralThread. **M.P. Karlsson:** A. Employment/Salary (full or part-time):; SpikeGadgets. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SpikeGadgets, NeuralThread. **K. Lee:** None. **A. Papadopoulou:** None. **C. Tindall:** None. **L.M. Frank:** F. Consulting Fees (e.g., advisory boards); NeuralThread. **K.E. Bouchard:** None. **P. Denes:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.02

Topic: I.04. Physiological Methods

Support: Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health (K12HD073945, AY)
National Eye Institute (R01EY028753, RKW)
Washington National Primate Research Center (P51 OD010425)
University of Washington Royalty Research Fund
National Science Foundation Graduate Research Fellowship (KK)
Big Data for Genomics and Neuroscience Training Grant (JB)
Center for Neurotechnology (EEC-1028725, DG)

Title: A toolbox to study cortical physiology in primates

Authors: *K. KHATEEB[1], J. BLOCH[1], J. ZHOU[1], M. RAHIMI[1], D. J. GRIGGS[2], M. LE[1], V. KHARAZIA[4], R. K. WANG[1], A. YAZDAN-SHAHMORAD[3];
[2]Electrical and Computer Engin., [3]Bioengineering and Electrical Engin., [1]Univ. of Washington, Seattle, WA; [4]Ernest Gallo Clin. and Res. Ctr., Emeryville, CA

Abstract: The primate cortex is a highly complex structure responsible for a variety of sophisticated tasks and behaviors including facilitating long-term memory storage, sensation, movement, and vision. Historically, lesioning and stimulation studies have been crucial for

elucidating the functions of broad cortical regions. However, little is understood with respect to the underlying cortical physiology and dynamics following injury or perturbation. To allow for clinical translation, it is vital that these phenomena are studied in a model sharing a high degree of complexity and evolutionary history with human cortex: the non-human primate (NHP) cortex. Such studies would allow for the development of improved therapies for neurological disorders. However, commonly employed tools for probing cortical physiology in NHP pre-clinical studies often require great surgical skill, produce variable results, and lack adaptability with other advanced tools. To address these challenges and accelerate the development of novel therapies, we developed a versatile toolbox for studying NHP cortical physiology. First, we demonstrated the photothrombotic technique, wherein intravenous infusion of a photosensitive dye, Rose Bengal, followed by targeted cortical illumination leads to the formation of localized thrombi in the vasculature. With this technique, we induced ischemic lesions extending through all layers of sensorimotor cortex in 7 adult macaques. Complementarily, we created a computational model to predict lesion sizes based on illumination parameters. Neuronal death in illuminated areas was validated and lesion sizes were measured via histological staining. With this toolbox, blood flow in the cortical microvasculature were monitored using optical coherence tomography angiography (OCTA) imaging, enabling in vivo lesion validation. Additionally, we incorporated a semi-transparent electrocorticography array to record and stimulate neural activity as lesions developed. The transparency of the array enabled illumination through the array to induce lesions, acquire OCTA images, and subsequently identify electrodes corresponding with ischemic areas in vivo. Thus, we observed the dynamics of the underlying neural activity before, during and after lesioning and stimulation. The tools presented here are compatible with previously established interfaces offering large-scale access for optical and electrical stimulation as well as imaging. In combination with behavioral findings, this toolbox can provide critical pre-clinical insights into fundamental cortical functions and drive the development of novel therapies for neurological disorders.

Disclosures: K. Khateeb: None. **J. Bloch:** None. **J. Zhou:** None. **M. Rahimi:** None. **D.J. Griggs:** None. **M. Le:** None. **V. Kharazia:** None. **R.K. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Washington, Oregon Health and Science University. F. Consulting Fees (e.g., advisory boards); Carl Zeiss Meditec. **A. Yazdan-Shahmorad:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.03

Topic: I.04. Physiological Methods

Support: Arnold O. Beckman Postdoctoral Fellowship Award

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
Larry L. Hillblom Foundation
National Institutes of Health grant number K08AG058749, R01NS100440, R01AG062196, UCOP-MRP-17-454755
Alzheimer Nederland
Swiss National Science Foundation Early Postdoc Mobility and Postdoc Mobility grants P2ZHP3-174753 and P400PB-186800

Title: Intrinsic network activity in human brain organoids

Authors: *T. SHARF[1], T. VAN DER MOLEN[1], E. GUZMAN[1], S. GLASAUER[1], A. BUCCINO[2], G. LUNA[1], Z. CHENG[1], M. AUDOUARD[1], K. RANASINGHE[3], K. KUDO[3], S. NAGARAJAN[3], K. TOVAR[1], L. PETZOLD[1], A. HEIRLEMANN[2], P. HANSMA[1], K. KOSIK[1]; [1]Univ. of California Santa Barbara, Santa Barbara, CA; [2]ETH Zurich, Zurich, Switzerland; [3]Univ. of California San Francisco, San Francisco, CA

Abstract: Human brain organoids replicate much of the cellular diversity and developmental anatomy of the human brain. However, the physiological behavior of neuronal circuits within organoids remains relatively under-explored. With high-density CMOS microelectrode arrays (26,400 electrodes) and shank electrodes (960 electrodes), we probed broadband and three-dimensional spontaneous activity of human brain organoids. These recordings simultaneously captured local field potentials (LFPs) and single unit activity as determined by Kilosort2. From spiking activity, we estimated a directed functional connectivity graph of synchronous neural network activity which showed a large number of weak functional connections enmeshed within a network skeleton of significantly fewer strong connections. Increasing the intrinsic inhibitory tone with a benzodiazepine altered the functional network graph of the organoid by altering the distribution of firing rates along a non-linear scale that produced a characteristic drug signature. Simultaneously examining the spontaneous LFPs and their phase alignment to spiking showed that spike bursts were coherent with theta oscillations in the LFPs. Our results demonstrate that human brain organoids have self-organized neuronal assemblies of sufficient size, cellular orientation, and functional connectivity to co-activate and generate field potentials from their collective transmembrane currents that phase-lock to spiking activity. These results point to the potential of brain organoids for the study of neuropsychiatric diseases, drug mechanisms, and the effects of external stimuli upon neuronal networks.

Disclosures: T. Sharf: None. **T. van der Molen:** None. **E. Guzman:** None. **S. Glasauer:** None. **A. Buccino:** None. **G. Luna:** None. **Z. Cheng:** None. **M. Audouard:** None. **K. Ranasinghe:** None. **K. Kudo:** None. **S. Nagarajan:** None. **K. Tovar:** None. **L. Petzold:** None. **A. Heirlemann:** None. **P. Hansma:** None. **K. Kosik:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.04

Topic: I.04. Physiological Methods

Title: Protocols for acute head-fixed recordings in awake mice with multiple Neuropixels probes

Authors: *T. K. RAMIREZ, G. R. HELLER, C. BENNETT, P. A. GROBLEWSKI, A. WILLIFORD, J. LUVIANO, S. DURAND, J. H. SIEGLE, S. R. OLSEN;
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Abstract: Recent years have seen a substantial leap forward in extracellular electrophysiology with the development and widespread distribution of Neuropixels probes (Jun et al., 2017). These probes' high electrode density (100 sites/mm) and long length (10 mm) make it possible to record from thousands of neurons simultaneously across dozens of brain regions, especially when multiple probes are used in parallel. However, producing stable recordings from multiple probes presents several experimental challenges, including brain stabilization and precise targeting. Here we present methods for reliable recordings in head-fixed awake mice using multiple acutely inserted Neuropixels probes. First, mice are implanted with a headframe and a removable glass window. This window allows optical access for mapping the boundaries of cortical visual areas using intrinsic signal imaging. Once the visual areas of interest are defined, we employ a set of targeting procedures to plan the insertion path for each Neuropixels probe. Our goal is to precisely target retinotopically matched locations in primary and higher-order visual areas (Juavinett et al., 2017). Our protocol employs techniques for brain stabilization which are a key component for successful recording sessions. To this end, on the day of the recording, we remove the glass window and replace it with a perforated plastic window ("insertion window") that allows the probes to be inserted into the brain. An agarose mixture is injected under the insertion window to stabilize the brain. This results in reduced brain movement and probe drift, allowing us to obtain stable recordings for over two hours with six Neuropixels probes. We have optimized this procedure for recording from the visual system, but it can be adapted to other brain regions and recording configurations. We describe our protocol steps in detail and share our experience with troubleshooting these powerful multi-probe recordings in the mouse brain.

Disclosures: T.K. Ramirez: None. **G.R. Heller:** None. **C. Bennett:** None. **P.A. Groblewski:** None. **A. Williford:** None. **J. Luviano:** None. **S. Durand:** None. **J.H. Siegle:** None. **S.R. Olsen:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.05

Topic: I.04. Physiological Methods

Support: NINDS intramural program

Title: Functional modules in local networks of the human temporal lobe

Authors: *J. I. CHAPETON[1], J. H. WITTIG, JR[1], S. K. INATI[2], K. A. ZAGHLOUL[1];
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Abstract: A fundamental question in neuroscience is how brain circuits and networks are organized. One hypothesis is that neural networks are modular and consist of groups of nodes that have denser connectivity to each other than to the rest of the network. Networks organized into spatially compact modules possess evolutionary, metabolic, and computational advantages, and can produce complex dynamics that may be particularly relevant for the human brain. Functional and structural modules have been identified in the primary sensory cortices of many mammals. In the human brain, fMRI has been used to identify modules in large scale brain networks such as the fusiform face area, and high magnetic field experiments have successfully identified orientation columns. However, direct evidence that higher-order regions of the human cortex are organized into functional modules at the sub-millimeter scale of local circuits has been lacking.

The strongest evidence that neural circuits are organized in a modular manner arises when measures of connectivity, structure, and function converge. A prime example of such convergence is the rodent barrel cortex in which neural circuits within individual cortical columns encode information about individual whiskers, and excitatory synaptic connections are typically constrained to neurons within the same barrel. Here, we map local functional connectivity as well as quantify the responses of individual neurons during an image classification task to show that the human anterior temporal lobe, a higher order brain region involved in semantic processing, exhibits a modular composition.

We use local field potential recordings and single unit spiking activity captured from microelectrode arrays in order to probe connectivity and functional significance at the sub-millimeter scale. We find that local networks in the anterior temporal lobe are composed of spatially compact and temporally persistent modules with a diameter of about 1.3mm. Information regarding image category is shared between neurons that are members of the same module, but largely independent across neurons belonging to different modules. Together, our results demonstrate that local circuits in the human temporal lobe can be naturally partitioned into modules based on functional connectivity, and that these modules exhibit neural responses that render them functionally distinct.

Disclosures: J.I. Chapeton: None. **J.H. Wittig:** None. **S.K. Inati:** None. **K.A. Zaghloul:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.06

Topic: I.04. Physiological Methods

Support: NIH grant MH081152
NIH grant MH090297

Title: Long-lived organotypic slice culture model of rat basolateral amygdala.

Authors: S. D. MICHAELSON[1], T. M. MULLER[1], M. BOMPOLAKI[2], A. MIRANDA TAPIA[1], H. SILVEIRA VILLARROEL[1], J. P. MACKAY[1], P. J. BALOGUN[1], J. H. URBAN[2], *W. F. COLMERS[1];
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Abstract: Our recent work has focused on the development of a behavioral stress resilience induced in male rats by repeated daily injections of Neuropeptide Y (NPY) into the basolateral amygdala (BLA). The resilience develops over time and persists to at least 8 weeks after NPY injections. To help unravel the underlying cellular mechanisms, we have developed and validated *in vitro* organotypic slice cultures (OTC) of BLA to identify neural mechanisms underlying NPY-induced stress resilience (Michaelson et al, J Neurosci. 40: 3231-3249). Originally developed in the early 1980s, OTCs have proved extremely useful for studying neural systems such as the hypothalamus, hippocampus and spinal cord. We have developed a model OTC of BLA tissue which maintains the electrophysiological and anatomical properties of BLA neurons *in vivo*. Rat BLA continues to develop until postnatal (P) day 28, but only OTCs prepared from younger (P14-P16) rats remained consistently viable to equivalent postnatal (EP) ages P70-P84; cultures prepared from male and female rats are equally viable. Principal neurons (PNs) in BLA OTCs (EP70-P84) were electrophysiologically similar to acute neurons from older (P70-P84) rats, though considerably smaller, and electrically more compact. Repeated daily treatment of OTCs (EP~42) with NPY resulted predominantly in reduced PN whole-cell capacitance, which correlated with reductions quantified in PN dendritic length and dendritic spine number in reconstructions of neurobiotin-filled cells. Conversely, treatment with the stress hormone, corticotropin-releasing factor (CRF), resulted in increased dendritic extent and spine density, consistent with increases in dendrite length and spine density observed in stressed animals. Pharmacological studies in OTCs unexpectedly showed the NPY5 receptor to underlie these structural changes. Subsequent *in vivo* experiments confirmed the morphological, pharmacological and behavioral consequences of NPY agonists on BLA neurons as was predicted in OTCs. BLA OTCs also permitted the determination of downstream signaling pathways for the Y5 receptor (calcineurin) and CRF (CaMKII) and showed that the effects of repeated NPY or CRF entirely reversed the effects of the other.
BLA OTCs have provided entirely unexpected, long-term physiological and pharmacological

results that were borne out *in vivo*, supporting the validity of BLA OTC as a model for BLA neurocircuitry.

Disclosures: S.D. Michaelson: None. **T.M. Muller:** None. **M. Bompolaki:** None. **A. Miranda Tapia:** None. **H. Silveira Villarroel:** None. **J.P. Mackay:** None. **P.J. Balogun:** None. **J.H. Urban:** None. **W.F. Colmers:** None.

Poster

P938. Electrophysiology Methods: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P938.07

Topic: I.04. Physiological Methods

Support: Schmidt Futures Foundation SF 857
NIH Grant R01MH120295
NSF 2034037
National Defense Science and Engineering Graduate Fellowship 00002116
NHGRI T32HG008345
Howard Hughes Medical Institute

Title: Light-weight Electrophysiology Hardware and Internet of Things Software Platform for Scalable, Cloud-Based Neural Recording Experiments

Authors: D. F. PARKS[1], K. VOITIUK[1], J. GENG[1], M. T. ELLIOTT[1], M. G. KEEFE[2], ***J. SEVETSON**[1], E. JUNG[1], A. ROBBINS[1], P. V. BAUDIN[1], V. T. LY[1], N. HAWTHORNE[1], D. YONG[1], S. E. SANSO[1], N. REZAEE[1], S. T. SEILER[1], C. PAZ FLORES[1], T. FINN[1], S. SANKAR[1], A. RAO[1], M. A. MOSTAJO-RADJI[1], R. CURRIE[1], K. B. HENGEN[3], D. B. FREEMAN[4], T. J. NOWAKOWSKI[2], A. POLLEN[2], S. R. SALAMA[1], M. TEODORESCU[1], D. HAUSSLER[1];
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Abstract: In vitro cell culture models of neural tissues are highly engineerable and scalable systems that are amenable to pairing with developments in robotic automation, microfluidics, and probes. Additionally, electrophysiological recordings are critical for the investigation and analysis of human neurological diseases.Currently, both cell culture experiments and electrophysiological recordings are limited by the need for technical expertise, expensive equipment, and material cost. This bottleneck limits broader influences that can be achieved by increasing sample throughput. Therefore, a massively scalable and cost efficient platform for tissue experiments, particularly experiments with functional readouts, would benefit genomics,

neuroscience, and translational medicine by enabling advanced high throughput tissue screens. To overcome limitations in equipment and cost, we leveraged two recent advances by our group. We developed an IoT architecture to allow horizontally scalable distance experiments. This architecture takes advantage of cloud computing tools to offer an online environment where researchers can run thousands of cell culture experiments at once.We also introduced an inexpensive neurophysiological recording platform (Piphys) that can be controlled through that online environment. We connected a Raspberry Pi to an Intan bioamplifier to design a hardware expansion circuit board with software to enable voltage sampling and user interaction. This standalone system was validated with primary human neurons, showing reliability in collecting real-time neural activity.Finally, we demonstrate the potential for integrating these technologies with 3D brain organoids. Through this, we illustrate feasibility for the hardware modules and cloud software to remotely control neural recording experiments. We also demonstrate horizontal scalability, enabling long-term observations of neural activity at scale. More broadly, these technologies offer opportunities for democratizing access to advanced tissue models by reducing costs and enabling new avenues of high throughput research.

Disclosures: D.F. Parks: None. **K. Voitiuk:** None. **J. Geng:** None. **M.T. Elliott:** None. **M.G. Keefe:** None. **J. Sevetson:** None. **E. Jung:** None. **A. Robbins:** None. **P.V. Baudin:** None. **V.T. Ly:** None. **N. Hawthorne:** None. **D. Yong:** None. **S.E. Sanso:** None. **N. Rezaee:** None. **S.T. Seiler:** None. **C. Paz Flores:** None. **T. Finn:** None. **S. Sankar:** None. **A. Rao:** None. **M.A. Mostajo-Radji:** None. **R. Currie:** None. **K.B. Hengen:** None. **T.J. Nowakowski:** None. **S.R. Salama:** None. **M. Teodorescu:** None. **D. Haussler:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.01

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

Title: Metastable dynamics during decision-making in mouse gustatory cortex

Authors: *L. LANG, G. LA CAMERA, A. FONTANINI;
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Abstract: Recent evidence suggests mouse gustatory cortex (GC) is involved in taste-guided decision-making in addition to sensory processing. Existing work has shown rodent GC exhibits metastable ensemble dynamics during ongoing and stimulus-evoked activity, but how these dynamics might evolve in the context of a taste-based decision-making task remains unclear. Here we employed analytical and modeling approaches to i) extract metastable dynamics in ensemble spiking activity from GC of mice performing a perceptual decision-making task; ii) investigate the computational mechanisms underlying metastability in this task. To extract

metastable dynamics, we applied Hidden Markov Model (HMM) analysis to spiking data from mice performing a 2-alternative choice task. In this task, 4 tastants cue correct licking directions according to the rule: Sucrose or Quinine → Lick Left; Maltose or Sucrose Octaacetate → Lick Right. HMMs were fit to ensemble spiking activity recorded in GC of mice performing the task at criterion. Decoded hidden states from HMMs were classified into categories according to differential frequencies of occurrence between trials of opposite type (e.g. Sweet cue vs. Bitter cue, Left cue vs. Right cue, Left action vs. Right action). We analyzed within-trial onset times of these states among the different categories and found that states related to taste information ('Quality-coding') preceded those related to cued directions ('Cue-coding'), which preceded those related to actions ('Action-coding'). This result is consistent with a view of neural activity evolving from sensory processing to abstract cue representation to decision and action in this task. To investigate computational mechanisms underlying this phenomenon, we modeled GC with a metastable spiking neural network (SNN) able to represent key task variables (stimuli, cued directions, and actions). The salient model features were clustered connectivity of excitation and inhibition, partially overlapping clusters, and a gating preparatory signal. Performing the same HMM analysis on SNN-generated data yielded coding state onset time distributions similar to those resulting from experimental data, which suggests our model captures the essence of GC's dynamics during perceptual decision-making. We conclude that activity in GC during perceptual decision-making is metastable, and that this metastability may serve as a substrate for sequentially encoding sensory, abstract cue, and decision information over time. Specific manipulations of the model may indicate aspects crucial for information processing and shed light on circuit-level mechanisms for coding via metastability.

Disclosures: L. Lang: None. **G. La Camera:** None. **A. Fontanini:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.02

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

Title: A model of electrical stimulation of a cortex-like phase-oscillator sheet

Authors: *T. FRACZEK[1], M. WILLY[2], Y. LIN[3], B. FLETCHER[4], H. J. CHIZECK[5], J. A. HERRON[6], E. T. SHEA-BROWN[2];
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Abstract: Phase oscillator-based models are increasingly investigated to model neural interactions across large brain regions. This modeling method is more computationally approachable than raw biophysical systems, while maintaining reproducibility of biologically

relevant behavior. However, no consistent method is proposed to relate the results of these models to empirical brain data. One method of investigating this relationship is to study the response of this model to realistic electrical stimulation for eventual comparison to real brain data.

We propose a model of electrical stimulation applied to a cortical sheet. The cortical sheet model, originally proposed by Breakspear et. al. is an extension of the Kuramoto model. This system describes individual phase oscillators, each representing a cortical column, embedded in physical space. The coupling function is based on the phase oscillator reduction of the Hodgkin Huxley model. Coupling strength is modulated by a spatial kernel, representing both transmission delays and a diminished coupling strength with distance. Our extension to this spatial phase oscillator model adds periodically stimulating electrodes at several locations on the cortical sheet. We then represent the effect of electrical stimulation on the sheet considering the known conductance properties of brain tissues. This may be used to study the responses of the model to realistic perturbations and track how cortical regions close to the electrode affect regions further away. In contrast to previous phase oscillator models of stimulation, such as those used to predict the effects of deep brain stimulation, our approach allows us to investigate how the effect of stimulation propagates.

We find that the behavior depends on the specific oscillators' coupling function parameters, the geometry of the stimulating electrodes, and the stimulation frequency relative to the oscillators' natural frequencies. Exploring combinations of these variable values, we find stimulation can entrain periodic ripples that propagate across the sheet, collapse all oscillators into a phase-locked state, or prevent phase locking altogether.

Future work will implement a similar model for sensing local field potentials, such as an ECoG electrode. Combined, these models may provide insight into medically relevant, large-scale models of electrical stimulation.

Disclosures: T. Fraczek: None. **M. Willy:** A. Employment/Salary (full or part-time):; DuPont specialty products, LLC. **Y. Lin:** None. **B. Fletcher:** None. **H.J. Chizeck:** None. **J.A. Herron:** None. **E.T. Shea-Brown:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

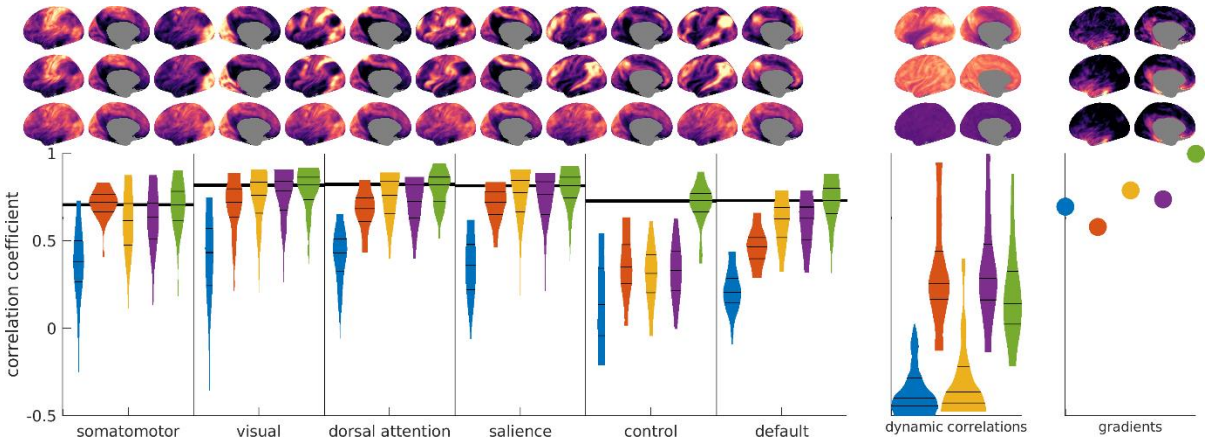
Program #/Poster #: P960.03

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

Title: Scalable unified analysis in human systems neuroscience

Authors: A. NANDA[1], **M. RUBINOV**[2];
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Abstract: A basic aim of science is to find truer models of observed reality. But because the ground truth is unknown and inaccessible, models can only be truer relative to rival models. Science makes progress when it selects progressively truer models relative to progressively stronger rivals. Model comparison drives this important process. It does so, in large part, through reducing model specification bias, by guarding against the selection of overly complicated or overly simple models. In practice, specification of rival models constrained by competing explanatory features defines data distributions. Unbiased sampling from these distributions generates surrogate data, and in this way allows to select between competing models. In this work, we will present results that use this approach to evaluate fundamental organizational principles of human neuroimaging networks. We will specifically present several interpretable models of whole-brain voxel-resolution activity that account for much of the structure of the default network, dynamic functional connectivity, architectural gradients and other higher-order phenomena. These results illustrate how rigorous model comparisons can reduce redundant explanations, clarify explanatory gaps and drive progress in human systems neuroscience.



Disclosures: A. Nanda: None. **M. Rubinov:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.04

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

Title: Modeling Cortical Cholinergic Reversal of Network Behavior induced by Synaptic Effects of Anesthesia

Authors: *B. ENIWAYE[1], V. BOOTH[2], A. G. HUDETZ[3], M. R. ZOCHOWSKI[4];
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Abstract: General anesthesics are typically used in the clinical setting to produce sedation and loss of consciousness. A variety of anesthetic agents can be used to reach similar behavioral end points while invoking different synaptic mechanisms. The administration of common inhalation anesthetics generally leads to changes in EPSPs and IPSPs associated with synaptic effects of glutamatergic and GABAergic neurotransmitters, respectively (Rudolph & Antkowiak, 2004) Recently, it was shown that intracerebral administration of acetylcholine (ACh) agonists can elicit wake-like behavior in the continued presence of an inhalational anesthetic in rodents (Mateos-Aparicio, 2020; Pal et al. 2018We focus on understanding the neuronal mechanisms responsible for the pharmacological reversal of anesthetic state by developing a computational neuron network model that captures the changes in neural firing behavior in response to ACh as well as the anesthetic-induced changes in postsynaptic potentials. We used a genetic algorithm to optimize model parameters to fit experimentally measured changes in firing behavior in the rat visual cortex *in vivo* in response to the application of the anesthetic desflurane. We then simulated the reversal of network connectivity and firing dynamics for increases in ACh concentration via its effect on the slow conductance potassium current. Results suggest that ACh-induced recovery from anesthesia may be related to increased neuronal excitability in response to ACh. Interestingly, the reversal of functional connectivity was incomplete, suggesting that cognitive function may remain impaired in the ACh reversal state.

Disclosures: B. Eniwaye: None. **V. Booth:** None. **A.G. Hudetz:** None. **M.R. Zochowski:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.05

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

Support: NIH NIBIB U01GM104604
NIH NIBIB U01EB025830
ARO W911NF2110091

Title: Towards a functional evaluation of memory recall performance in a large-scale neuronal network model of hippocampus: How the recall quality of a CA3 network scales with increasing complexity of network topology and synaptic mechanisms

Authors: *Z. CHOU[1], G. J. YU[1], J.-M. C. BOUTEILLER[2], T. W. BERGER[2];
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Abstract: Our previous work in developing a large-scale, spiking neuronal network model of hippocampus has shown that large-scale simulations have the potential to capture emergent network dynamics such as spatiotemporal clustering of spiking activity. However, we currently lack a direct way of measuring the functional significance of these emergent phenomena, namely how they affect the role that the hippocampus plays in memory. In this work, we adapt an experimental paradigm that quantifies recall quality of a network via several cycles of storage and retrieval of cue patterns (Cutsuridis et al., 2009) to a CA3 microcircuit with the ultimate goal of examining how recall quality, and consequently storage capacity, in the CA3 is influenced at the large-scale. The CA3 network consists of biophysically detailed compartmental morphologies of pyramidal cells and interneurons, and receives mossy fiber inputs from granule cells, entorhinal cortex projections, and associational inputs from recurrent connections. The number of interneuron types incorporated into the network (basket, axo-axonic, OLM) was modified while normalizing their inhibition to the pyramidal cells to isolate the effects of the change in network topology. The storage capacity of the CA3 model was then characterized by generating a "forgetting" curve by measuring the decrease in recall quality as an increasing number of patterns was stored in the same network. We repeated these evaluations at small (n=100), medium (n=1000), and large (n=100,000) network sizes to compare the results at different scales. Finally, the STDP learning rule for potentiation was modified from an additive rule to a log-normal STDP update function (Gilson and Fukai, 2011) to examine the influence of more complex synaptic mechanisms on recall quality. This preliminary work in the CA3 microcircuit provides a step towards evaluating memory recall in a large-scale simulation of the entire hippocampus. It may also constitute a useful platform to investigate the effects of multiple parameters on memory recall performance, allowing the simulation of not only the changes taking place in neurodegeneration but also the effects of novel drug therapies.

Disclosures: Z. Chou: None. **G.J. Yu:** None. **J.C. Bouteiller:** None. **T.W. Berger:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.06

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

Title: Recording and clustering action potentials from the surface of the human brain using conformable, organic, high spatiotemporal resolution neural interface devices

Authors: *A. HASSAN[1], Z. ZHAO[2], S. A. SHETH[3], D. KHODAGHOLY[2], J. GELINAS[4]; [1]Biomed. Engin., [2]Electrical Engin., Columbia Univ., New York, NY; [3]Neurosurg., Baylor Col. of Med., Houston, TX; [4]Columbia Univ. Med. Ctr., New York, NY

Abstract: Recording neurophysiological signals at the spatiotemporal resolution of action potentials is a fundamental step to understand the mechanisms underlying diverse brain functions, but acquiring such data from the human brain is challenging due to restricted spatial resolution and invasiveness of currently available technologies. We developed an organic mixed-conducting particulate composite material (MCP) to establish a scalable and biocompatible interface between soft and hard electronics. MCP enabled integration with conducting polymer-based electrode arrays (NeuroGrids) and creation of neural interface devices that can be placed on the surface of the human through small craniotomies and burr holes. These devices recorded both actional potentials and local field potentials from superficial cortical neurons of human subjects without penetrating the brain surface (n = 64 channels per array). Subsequently, we performed spike sorting followed by cluster-cutting to identify putative single-units. We then assessed the effect of the electrodes' spatial distribution on the quality metrics of clustered units. We identified 229 putative single-units (85 pyramidal cells, 144 interneurons; n = 7 subjects). Action potential detection was strongly dependent upon length of recording and noise floor. Neurons showed consistent modulation and phase-locking in the spindle band, and we also identified reciprocal monosynaptic interactions between putative pyramidal cells and fast-spiking interneurons. When action potential waveforms were detected across multiple channels, deliberate elimination of neighboring channels (mimicking access to only a single electrode) was associated with loss of detection or decreased isolation distance, increased L-ratio, and increased contamination rate. These results suggest that MCP- and NeuroGrid-based neural interface devices are capable of acquiring neurophysiological signals at the resolution of individual neurons from the surface of the human brain. Spatial configuration of surface electrodes that permits acquisition of a single action potential waveform on multiple channels increases yield and quality of single-units, with key ramifications for design of human neural interface devices.

Disclosures: A. Hassan: None. **Z. Zhao:** None. **S.A. Sheth:** None. **D. Khodagholy:** None. **J. Gelinas:** None.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.07

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

Support: SSN MH122023

Title: Deep architectures for predicting in vivo local field potential

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Abstract: Deep Architectures for Predicting In Vivo Local Field Potential Matthew Carroll[1], Ziao Chen[1], Drew B. Headley[2], Satish S. Nair[11]Electrical Engineering & Computer Science Dept., University of Missouri, Columbia, MO[2]Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ**Abstract** Local field potential (LFP) measurements are widely used in neuroscience to characterize the interactions among neurons. This is because the temporal synchronization of the membrane potentials of principal neurons (PNs) is partly reflected in oscillations of the LFP. These oscillations are thought to be relevant to the computations carried out by networks of neurons, and recently methods have been developed to modulate them using closed-loop stimulation conditioned on the LFP signal. Since closed-loop approaches have to be run in real-time, their accuracy and precision for targeting specific events in the LFP is limited to relying purely on past values. Towards overcoming this, the present study explores the capabilities of statistical learning models in predicting future values of the LFP for enhancing the efficacy of closed-loop control. We focus on predicting the temporal properties of transient gamma-band activity (30-80 Hz) using online in vivo LFP measurements from behaving rodents. Specifically, on predicting the amplitude of future transient burst cycles in the LFP using past values with statistical learning models.
As a first step, we used LFP from a physiologically-based computational model of a rodent amygdala that matched the in vivo LFP characteristics. In particular, it matched the transient gamma bursts which occur over brief durations of 3-4 cycles, which present exceptional challenges to closed-loop detection. A comparison of the various types of networks to predict future model LFP values is shown in the table below. In comparison to the filtered LFP, accuracy of raw LFP prediction using simple architectures was limited to the 1 to 5 ms horizon. However, future values of a 4^{th} order band-pass filtered LFP output were predicted well using 50 ms of raw LFP values are input with comparable performance among the networks.
Entries list mean-square error (R^2) values
Adding the firing rate of interneurons as another input feature did improve the prediction and increase the R^2 values for the 40 ms and 50 ms cases to .51 and .23, respectively. Ongoing investigation of the underlying mechanisms showed that suitable architectures could learn both the dynamics of the filter as well as the oscillations. The next step will be to explore XAI architectures to ascertain the salient features that could predict future values of the LFP.

Disclosures: M. Carroll: A. Employment/Salary (full or part-time):; University of Missouri. **Z. Chen:** A. Employment/Salary (full or part-time):; University of Missouri. **D.B. Headley:** A. Employment/Salary (full or part-time):; Rutgers University. **S.S. Nair:** A. Employment/Salary (full or part-time):; University of Missouri.

Poster

P960. Experimental Computational Tools: Neural Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P960.08

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

Support: NIH Grant U19-NS104590-01
NSF Award 1811597
NSF LRAC allocation BIR20001 on TACC Frontera

Title: Scalable parallel data management for large-scale computational models of neuronal networks

Authors: *I. RAIKOV[1], A. D. MILSTEIN[3], P. MOOLCHAND[2], D. HADJIABADI[1], A. CHATZIKALYMNIOU[2], I. SOLTESZ[1];
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Abstract: Large-scale computational models of the brain are necessary to accurately represent anatomical and functional variability in neuronal biophysics across brain regions and also to capture and study local and global interactions between neuronal populations on a behaviorally-relevant spatial scale.
We present a flexible HDF5-based data format for storing morphological, synaptic, and connectivity information of large neuronal network models, and an accompanying open-source software library that provides efficient, scalable parallel storage and MPI-based data movement capabilities. The set of operations provided by the data management library includes scalable routines for iterating over data elements associated with an individual model cell, parallel data read and write, parallel map and reduce, and data generator routines that enable the iterative processing of data sets that are too large to fit in memory. This set of operations facilitates common functionality for construction, simulation and analysis of computational neuronal network models at a scale of millions of neurons on high-performance computing systems, while still permitting analysis and simulations of parts of the model on single computers.
We highlight the use of the data management library for a highly scalable infrastructure for simulation, optimization, and analysis of large-scale models of neuronal networks comprised of detailed biophysical models of diverse neuron types, based on the NEURON simulator. The functionality thus offered is centered on several core principles that are crucial for the development large-scale neural modeling systems:
1) reproducibility, or the ability to conduct simulation and analysis with consistent model parameters, such as synaptic placement and connectivity; 2) flexibility to allow the rapid iteration of scientific hypotheses about model structure on any level of the neural hierarchy; 3) scalability, or the ability to efficiently use a broad range of computing hardware, from single computers to large high-performance computing clusters. We demonstrate the use of this infrastructure, to construct, simulate and analyze a full-scale, anatomically and physiologically

detailed models of the dentate gyrus (DG) and CA1 regions in the rodent hippocampus that contain anatomically accurate numbers of neurons and synaptic connectivity.

Disclosures: I. Raikov: None. **A.D. Milstein:** None. **P. Moolchand:** None. **D. Hadjiabadi:** None. **A. Chatzikalymniou:** None. **I. Soltesz:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.01

Topic: B.11. Glial Mechanisms

Support: NSERC RGPIN-2021-03732
McMaster University startup funds

Title: In vivo investigation of astrocyte calcium activity in the mouse hypothalamic paraventricular nucleus

Authors: *K. SANDOVAL, J. RYCHLIK, K. Y. CHOE;
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Abstract: Neurons and astrocytes in the hypothalamic paraventricular nucleus (PVN) exist in a structurally compact microenvironment, where bidirectional communication between the two cell types influences the physiological and behavioural output of this brain region. Stress and social stimuli are both potent modulators of neuronal activity in the PVN. As a part of the hypothalamic-pituitary-adrenal axis, corticotropin-releasing hormone (CRH) neurons in the PVN are activated by stress-induced increase in noradrenaline levels (Bains et al., 2015). Social stimuli activate oxytocin neurons in the PVN to cause oxytocin neuromodulation of downstream brain regions, facilitating social interactions (Resedez et al., 2020). Recent *in vitro* experiments demonstrated that PVN CRH neurons dendritically release vasopressin in an activity-dependent manner, which activates V1a receptors in surrounding astrocytes to induce calcium (Ca^{2+}) responses (Chen et al., 2019). Another study showed that oxytocin, which can be released dendritically in response to social stimuli, activates oxytocin receptors in cultured hypothalamic astrocytes and triggers Ca^{2+} responses (Di Scala-Guenot et al., 1994). Based on these reported findings, we hypothesize that PVN astrocytes encode both stress and social stimuli as Ca^{2+} events *in vivo*. To examine this, we performed *in vivo* fiber photometry recordings in the mouse PVN with astrocyte-specific expression of GCaMP6, a genetically-encoded Ca^{2+} indicator (induced by AAV2/5-promGFAP-GCaMP6f). We recorded Ca^{2+} signals in the PVN of freely behaving mice while they were subjected to stressful stimuli (e.g., looming shadow) or during reciprocal social interaction with a novel stimulus mouse in a home cage setting. During these sessions, the behaviours of mice were also video-recorded and manually scored by an

independent observer. We found that PVN astrocytes exhibit a large spike in Ca^{2+} signal in response to stressful stimuli (z dF/F= >10). In contrast, social interactions induced smaller Ca^{2+} responses (z dF/F= ~1-2) that varied depending on the social stimulus type. Social touching by stimulus mouse reliably elevated the Ca^{2+} signal in experimental mice, while anogenital sniffing of the stimulus mouse reduced it. These demonstrations show that PVN astrocytes exhibit robust, stimulus-specific Ca^{2+} responses to both stress and social stimuli *in vivo*. To extend these findings, we are currently investigating whether these Ca^{2+} responses are dependent on the spiking activity of CRH and oxytocin neurons in the PVN, as well as their potential functional impact on PVN neuronal activity and behaviour.

Disclosures: K. Sandoval: None. **J. Rychlik:** None. **K.Y. Choe:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.02

Topic: B.11. Glial Mechanisms

Title: Molecular clock synchronization among segregated neural populations

Authors: *L. GIANTOMASI, J. F. RIBEIRO, O. BARCA-MAYO, D. DE PIETRI TONELLI, L. BERDONDINI;
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Abstract: In mammals the suprachiasmatic nucleus (SCN) of the hypothalamus is considered the master circadian pacemaker, which coordinates circadian rhythms in the central nervous system (CNS) and across the entire body. The SCN receives light input from the eyes through the retinohypothalamic tract and then it synchronizes other clocks in the CNS and periphery, thus orchestrating rhythms throughout the body. However, little is known about how so many cellular clocks within and across brain circuits can be effectively synchronized.In this work we investigated the implication of two possible pathways: i) paracrine factors-mediated synchronization and ii) astrocytes-mediated synchronization. To study these pathways, we adopted an *in vitro* research model based on a lab-on-a-chip microfluidic device developed in our laboratory. This device allows growing and compartmentalizing distinct neural populations connected through a network of astrocytes or through a cell-free channel in which the diffusion of paracrine factors is allowed. By taking advantage of this device, we synchronized rat cortical neurons (Embryonic day 18) in one compartment and analyzed, in different experimental conditions, the induced expression of clock genes in a distant neural network grown in the second compartment. Results show that both pathways can be involved, but might have different roles. Neurons release factors that can diffuse to synchronize a neuronal population. The same factors can also synchronize astrocytes that, in turn, can transmit astrocyte-mediated molecular

clocks to more distant neuronal populations. Using microfluidic devices featuring different channel lengths, we found that paracrine factors-mediated synchronization occurs only in the case of a short distance between neuronal populations. On the contrary, interconnecting astrocytes define an active channel that can transfer molecular clocks to neural populations also at long distances. The study of possibly involved signaling factors indicate that paracrine factors-mediated synchronization occurs through GABA signaling, while astrocytes-mediated synchronization involves both GABA and glutamate.These findings strength the importance of the synergic regulation of clock genes among neurons and astrocytes, and suggest a previously unknown role of astrocytes as active cells in distributing signals to regulate the expression of clock genes in the brain.

Disclosures: L. Giantomasi: None. **J.F. Ribeiro:** None. **O. Barca-Mayo:** None. **D. De Pietri Tonelli:** None. **L. Berdondini:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.03

Topic: B.11. Glial Mechanisms

Support: NINDS Grant 5R01NS102306

Title: Neuron-astrocyte interactions underlying noradrenergic modulation of wake-promoting vPAG^{DA} neurons

Authors: *N. PETERSEN[1], S. W. CENTANNI[1], G. J. SALIMANDO[1], K. E. MCCANN[2], M. A. STAVARACHE[3], D. WEINSHENKER[2], D. G. WINDER[1];
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Abstract: Sleep and arousal disorders are commonly comorbid with many psychiatric illnesses, however the basic underlying physiology governing wakefulness is not well known. Previous work has implicated a small population of dopamine (DA) neurons in the ventral periaqueductal gray (vPAG) as being important in arousal. Activation of these DA neurons (vPAG^{DA}) is sufficient to increase arousal, and lesioning or direct inactivation of these neurons decreases arousal. Recently, we delineated a novel arousal circuit from the noradrenergic locus coeruleus (LC) to the vPAG^{DA} neurons. Specific activation of G_q-coupled alpha$_1$-adrenergic receptors (α_1ARs) with phenylephrine increases excitatory drive onto vPAG^{DA} neurons and promotes arousal. α_1AR expression is enriched on vPAG astrocytes, and activation of astrocytic $G_q$signaling is sufficient to promote wakefulness. However, the mechanism of this neuron-astrocyte interaction is unknown. Activation of astrocytes is known to induce release of

extracellular “gliotransmitters”, including the purines ATP and adenosine. We hypothesize that purinergic transmission via ATP from neighboring astrocytes mediates noradrenergic modulation of $vPAG^{DA}$ neurons. Application of 3μM phenylephrine in *ex vivo* mouse brain slices significantly increases calcium transients in $vPAG^{DA}$ neurons of TH-Cre mice expressing the fluorescent calcium indicator GCaMP7f. In parallel, phenylephrine also increased the frequency of calcium oscillations in GCaMP7s expressing astrocytes in the vPAG. In TH-Cre mice injected with a viral mixture of an astrocyte-specific Gq-DREADD (AAV5-GfaABC1D-hM3Dq) and Cre-dependent GCaMP7f (AAV9-Syn-FLEX-GCaMP7f) into the vPAG, direct activation of astrocyte G_q signaling significantly increases calcium transients in $vPAG^{DA}$ neurons, suggesting astrocytic modulation of these cells. Additionally, whole-cell recordings from these cells reveal that antagonism of purinergic P2X ligand-gated ion channels with PPADS or TNP-ATP followed by co-application of phenylephrine prevents the increase in excitatory drive onto $vPAG^{DA}$ neurons via α_1AR activation. Together, these data suggest that vPAG astrocytes may play an important role in mediating arousal via regulation of wake-promoting $vPAG^{DA}$ neuron activity by way of extracellular ATP release. Future experiments will further test our hypothesis with the goal of elucidating the synaptic mechanisms underlying noradrenergic modulation of $vPAG^{DA}$neurons.

Disclosures: N. Petersen: None. **S.W. Centanni:** None. **G.J. Salimando:** None. **K.E. McCann:** None. **M.A. Stavarache:** None. **D. Weinshenker:** None. **D.G. Winder:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.04

Topic: B.11. Glial Mechanisms

Support: NIH EY021222
NIH EY030568

Title: Sonic hedgehog signaling as an intermediary between retinal inputs and astrocytes for the recruitment of GABAergic interneurons

Authors: *R. SOMAIYA[1,4], A. GARCIA[5,6], M. A. FOX[4,2,3,7];
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Abstract: Visual thalamus receives direct inputs from retinal ganglion cell (RGC) axons and is important for both image-forming and nonimage-forming visual functions. Recent studies have shown that these RGC inputs play important roles in the development of cell types and circuits in these regions. For example, retinal inputs regulate the long-distance recruitment of GABAergic interneurons into two regions of visual thalamus - the dorsal and ventral lateral geniculate nuclei. Our lab recently discovered that these retinal inputs induce astrocytes to generate fibroblast growth factor 15 (FGF15), a potent motogen that is essential for interneuron migration into these regions. However, how retinal inputs induce the astrocytic expression of FGF15 remains unresolved. Here, we tested the role of RGC-derived Sonic Hedgehog (SHH) in this process since RGCs are known to release SHH in the brain and SHH is known to induce FGF15 expression in other parts of the embryonic brain. Using transcriptomic analysis, *in situ* hybridization, and reporter line, we observed that thalamus-projecting RGCs express SHH, and SHH receptors and signaling pathway components are expressed in the developing visual thalamus. Specifically, we discovered that thalamic astrocytes express the downstream components of SHH-signaling such as Patched-1, Smoothened, and Glioma-associated oncogene transcription factors. To assess the importance of this pathway in developing visual thalamus, we next generated a conditional mutant mouse line that lacks SHH specifically from RGCs. In thalamic sections of these mutants, as compared to littermate controls, we observed a significant decrease in both *Fgf15* mRNA expression and GABAergic interneuron number. Thus, these data identify a novel axo-glial signaling pathway in the development of visual thalamus and shed light on new ways in which astrocytes mediate the recruitment of interneurons into the developing visual thalamus.

Disclosures: R. Somaiya: None. **A. Garcia:** None. **M.A. Fox:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.05

Topic: A.07. Developmental Disorders

Title: Identification of cellular and pathway targets for treatment in post hemorrhagic hydrocephalus

Authors: C. ADEYEMI, ***K. ABDI**;
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Abstract: Post hemorrhagic hydrocephalus (PHH) is a prevalent condition leading to increased morbidity and disability related to neurocognitive impairment and motor dysfunction. Identification of target-able cells, pathways, and effective drug compounds that can treat PHH are needed, since the current standard of care involving shunts fail at a high rate and contain

many drawbacks for patients. Frequently observed damage to the brain ventricular lining and an abundance of genetic evidence points to multiciliated ependymal cells as an important mediator of hydrocephalus etiology. Here we identify that acute exposure to the inflammatory cytokine TNF-a during the developmental phase of radial glial progenitors derails their programming into multiciliated ependymal cells. A 48 hour exposure to TNF-a blocked upregulation of Foxj1, Multicilin, and c-Myb and dramatically reduced the number of multiciliated ependymal cells in primary cultures. We found that while Notch pathway is downregulated during normal development of progenitors into multiciliated cells, this pathway remained highly elevated during TNF-a exposure. Co-incubation of radial glial progenitors with the notch inhibitor, DBZ, and TNF-a completely rescued the number of multiciliated ependymal cells produced and enhanced the expression of Multicilin, Foxj1, and c-Myb above untreated levels. Additionally, the same rescue could be achieved with DBZ added after the acute exposure to TNF-a, potentially offering broader time window to treat PHH. Since multiciliated ependymal cells are essential to the proper flow of cerebrospinal fluid in brain ventricles and their damage has readily been observed in PHH, we believe our study reveals a sensitive period for multiciliated cell development that could be derailed by inflammatory exposure and rescued by notch inhibition. This acute inflammatory exposure and the effect on developing multiciliated cells could underlie some of the mechanisms in PHH seen after intraventricular hemorrhage in newborns and preterm infants.

Disclosures: C. Adeyemi: None. **K. Abdi:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.06

Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH109706-02

Title: Functional interpretation of SARS-CoV-2 coding mutations in brain-derived organoids using variants discovered from sewage and clinical samples

Authors: *V. VO[1], R. GU[1], K. PAPP[2], C. CHANG[1], S. SHANG[1], H. BAKER[1], R. TILLETT[1], D. GERRITY[2], E. C. OH[1];
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Abstract: The decline in diagnostic testing for SARS-CoV-2 is expected to delay the tracking of COVID-19 variants in the United States and limit the interpretation of viral sequence variation in the brain. We hypothesize that wastewater surveillance programs, which are agnostic to clinical

testing, can provide an effective alternative for detecting emerging viral variants and an opportunity to interrogate functional consequences from these genotypes using brain-derived organoids. Here, we performed amplicon-based whole genome sequencing of SARS-CoV-2 for 86 wastewater samples in Southern Nevada and identified the B.1.1.7 and B.1.429 lineages in December 2020. Whole genome sequencing for both lineages in 575 clinical samples failed to identify the variants until January 2021, suggesting that pooled wastewater samples may expedite viral variant detection when sequencing of clinical samples is limited. During our investigations, we identified novel mutations in the coding genome of SARS-CoV-2. To evaluate the functional impact of viral variants, we infected brain-derived organoids and examined cell growth and proliferation status, as well as transcriptional and proteomic changes. Our data highlight novel modules that are altered in response to viral mutations and provide a mechanism for the association of SARS-CoV-2 infection and brain pathology in humans.

Disclosures: V. Vo: None. **R. Gu:** None. **K. Papp:** None. **C. Chang:** None. **S. Shang:** None. **H. Baker:** None. **R. Tillett:** None. **D. Gerrity:** None. **E.C. Oh:** None.

Poster

P037. Neural Development: Neuron-Glia Interactions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P037.07

Topic: E.04. Voluntary Movements

Support: CIHR
AAE
ICP

Title: Planar Sholl analysis of astroglial filaments in optically cleared thick tissue

Authors: R. ROOMES-DELPEACHE[1], O. AVIVI[3], J. LOPEZ GROSS[2], R. AKASAKA[4], M. ZANJIR[1], M. J. GASTINGER[5], *L. AVIVI-ARBER[1];
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Abstract: Neuronal and astroglial processes networks can span through multiple layers of the sensorimotor cortex. It is unknown whether injury, disease, as well as learning, can result in layer-dependent structural changes manifested as differential generation, degeneration, or regeneration of cellular processes, and reflected in differential changes in processes density within and across the cortical layers. Such changes may reflect or allow for altered cellular functions which can impact sensory-motor behaviors.
To quantify the differential density of astrocyte processes across multiple cortical layers, we developed a novel Imaris XTension implementing a planar modification of the spherical Sholl

Analysis. It quantifies the number of intersections of astroglial processes with multiple horizontal planes within the rat primary motor cortex.
Our XTension to Imaris software application (version 9.5.1) takes the 3D positions from an automatic tracing of all astroglial processes within motor cortex layer 1 of a 1 mm-thick brain tissue optically cleared with the CLARITY technique and subsequently immunolabeled with the astroglial marker glial fibrillary acidic protein (GFAP). It computes the distance to a plane generated from three user defined points. Spot objects are then created within the application to display all filament intersections with horizontal planes spanning through the cortex. The number and spacing of the planes can be defined by the user. The XTension then compares all intersection spots at once using matrix multiplication and categories and color-coded spots based on their distance from the original plane of interest. An error parameter is introduced to control underfitting issues that result from working with floating point numbers. Our novel XTension can work on 2D and 3D (planar and volumetric) datasets and is a useful tool to investigate layer-dependent structural differences or changes in cellular processes density.

Disclosures: R. Roomes-Delpeache: None. **O. Avivi:** None. **J. Lopez Gross:** None. **R. Akasaka:** None. **M. Zanjir:** None. **M.J. Gastinger:** Other; Oxford Instruments Imaris Biteplane technical Support. **L. Avivi-Arber:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.01

Topic: A.07. Developmental Disorders

Support: NIH Grant P20GM103408

Title: Dscaml1 shapes cerebellar granule cell dendrite length and complexity

Authors: R. KSHETRI[1], A. D. REMOLINA[1], A. KINDALL[2], P. G. FUERST[3], *B. D. RICHARDSON[1];
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Abstract: It is estimated that 1 in every 54 US children are diagnosed with autism spectrum disorder (ASD). Studies have indicated that the environmental and genetic factors have been implicated in the development of ASD, however the neural underpinnings of ASD are complex. Recently the cerebellum has gained more attention and been identified as one of the brain areas associated with ASD etiology. How the cerebellum contributes to ASD is unclear, but identifying how a loss of or mutation in ASD-linked genes alters cerebellar anatomy and function may be important to understanding this relationship. Loss of function mutations in the

dscam gene have been consistently identified in individuals with ASD and like disorders and its homolog, *dscaml1,* is expressed in cerebellar granule cells. The *dscaml1* gene encodes DSCAML1 protein that is important for the formation and maintenance of excitatory synapse and neuronal organization, but little is understood about how DSCAML1 impacts cerebellar GC anatomical and functional circuit properties, perturbations in which will impact cerebellar output and related motor and non-motor circuits or behaviors. To address our hypothesis that that DSCAML1 plays a significant role in GC dendritic development we evaluated the structure of cerebellar GC dendrites that receive excitatory input from mossy fibers. The purpose of this study is to determine DSCAML1-dependence of cerebellar GC dendritic development that is key to shaping signal integration in the cerebellar cortex. Our study used a mouse model which carries null Dscaml1 alleles generated by gene trap insertion in *dscaml1*. By using Golgi-Cox staining and 3D digital reconstruction of cerebellar granule neurons with Neurolucida software (MBF Biosciences), we assess the DSCAML1-dependence of cerebellar GC dendritic development in heterozygous (*dscaml1*$^{GT/+}$) and homozygous (*dscaml1*$^{GT/GT}$) *dscaml1* null mice. Our preliminary analysis of cerebellar GC dendrites revealed that the average dendritic length and dendritic complexity (branching) of homozygous DSCAML1 null mice was reduced relative to heterozygous mice. This finding indicates the possible role of DSCAML1 in cerebellar GC dendritic development and their receptive fields. This study will help to identify the roles for *dscaml1* in determining key components of cerebellar GC synaptic anatomy and physiology that may underpin both motor and non-motor behavioral phenotypes.

Disclosures: R. Kshetri: None. **A.D. Remolina:** None. **A. Kindall:** None. **P.G. Fuerst:** None. **B.D. Richardson:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.02

Topic: A.07. Developmental Disorders

Support: NIH Grant AG056850

Title: Abnormal microglial morphology and phagocytic function in Down syndrome developing brain

Authors: *I. RIVERA[1], M. LIOUDYNO[1], B. S. HSU[2], J. BUSCIGLIO[1];
[1]DEPARTMENT OF NEUROBIOLOGY AND BEHAVIOR, UNIVERSITY OF CALIFORNIA-IRVINE, Irvine, CA; [2]BIOLOGICAL SCIENCES, UNIVERSITY OF CALIFORNIA-IRVINE, IRVINE, CA

Abstract: As the primary immune cell of the mammalian CNS, microglia play an important role in maintaining normal brain structure and function. Our lab and collaborators have reported increased inflammatory molecule levels and altered microglia morphology in brains of children with Down syndrome (DS), a genetic disorder caused by the presence of a partial or fully triplicated copy of chromosome 21. However, much is still unknown regarding when microglia alterations emerge in DS brains. Thus, it is critical to identify the role of microglia in the neuroinflammatory process during prenatal and early postnatal DS brain development. Here, we explore microglia morphology and function in prenatal human cortical tissue. To characterize microglial morphology, we performed immunofluorescence (IF) staining and image analysis on euploid control and DS prenatal human cortical tissue using the microglia-specific marker IBA1. In euploid control prenatal cortical tissue, we observed only amoeboid (spherical) microglia, which characterizes the earliest stage of microglia in the developing mammalian brain. In contrast, in DS prenatal cortical tissue, we observed both amoeboid and intermediate stage microglia, exhibiting short processes. To evaluate microglial function, microglia cultures from control and DS prenatal cortical tissue suspensions were established and developed for 34 days *in vitro*. Phagocytic function was tested using pHrodo Zymosan A bioparticles and then the cultures were fixed, processed for IF, and labeled with IBA1. Interestingly, we observed that trisomic microglia displayed significantly increased particle engulfment per cell compared to euploid cultures. The results suggest that the early phenotypic alterations observed may represent an advanced developmental stage of DS microglia, raising the possibility of an accelerated microglial differentiation phenotype, consistent with the presence of a chronic neuroinflammatory process in DS brains. To further characterize prenatal DS microglia, microglial cells were isolated using Percoll density gradients and fluorescence-activated cell sorting (FACS). Ongoing experiments are aimed to establish the relationship between abnormal morphology and function and gene expression changes in DS prenatal microglia.

Disclosures: I. Rivera: None. **M. Lioudyno:** None. **B.S. Hsu:** None. **J. Busciglio:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.03

Topic: A.07. Developmental Disorders

Support: CIHR Grant PJT 153013

Title: Effects of the pro-cognitive agent RIV5061 in a mouse model of Down Syndrome

Authors: K. A. TRESIDDER, P. J. GAGOLEWICZ, R. H. MEHDER, R. D. ANDREW, *B. M. BENNETT;
Biomed. and Mol. Sci., Queens Univ., Kingston, ON, Canada

Abstract: Down syndrome (DS) is a leading genetic risk factor for early-onset Alzheimer's disease (AD), and there is a high incidence of AD in adults with DS. The current study assessed the effects of RIV5061 on behavioral, biochemical and electrophysiological properties of the *Dp(16)1Yey/+* (Dp16) mouse model of DS. RIV5061 is a novel dual pharmacophore with partial agonist activity at $GABA_A$ receptors combined with activation of NO/cGMP/CREB signalling. RIV5061 has been shown to have pro-cognitive, anti-inflammatory and disease-modifying effects in several mouse models of AD. Male and female Dp16 mice (2-4.5 months of age), along with their wild-type (WT) littermates, were administered either vehicle or 27 mg/kg/day of RIV5061 in the drinking water for 14 weeks, with an additional daily oral bolus of vehicle or 3 mg/kg of RIV5061. Behavioural analyses (Morris Water Maze, Y-Maze, and Light/Dark (L/D) Box) were performed prior to and following drug treatment. Upon conclusion of drug administration, we assessed the levels of a number of relevant protein markers in neocortical and hippocampal homogenates, CA3-CA1 long-term potentiation (LTP) in hippocampal slices, and spine density of dorsal hippocampal CA1 pyramidal cells.
At each time point assessed, control male Dp16 mice performed significantly worse than WT in the Y-maze and L/D box tasks ($p < 0.05$), indicating a deficit in spatial working memory and increased anxiety, respectively. These differences were not observed in control female mice. There were no differences in MWM task performance between control Dp16 and WT mice of either sex. RIV5061 did not affect performance in any of the behavioural tasks. Immunoblot analysis of a number of AD-relevant markers showed few differences between genotypes and following drug treatment, except for glial fibrillary acidic protein (GFAP), which was reduced following RIV5061 administration to both WT and Dp16 mice. Slice electrophysiology revealed no difference in LTP magnitude between control male Dp16 and WT mice, but RIV5061 did facilitate LTP induction in WT mice. Spine density in CA1 pyramidal cells from female Dp16 mice was significantly less than in WT mice; assessment of the effect of RIV5061 on spine density is ongoing.
Our results indicate that Dp16 mice up to 8-9 months of age do not display robust AD-like phenotypic changes compared with their WT counterparts. Consequently, the pro-cognitive and disease-modifying effects of RIV5061 seen in transgenic AD mouse models were not observed here - with the exception of an anti-inflammatory effect, as indicated by the reduction in the levels of GFAP, a marker for activated astrocytes.

Disclosures: K.A. Tresidder: None. **P.J. Gagolewicz:** None. **R.H. Mehder:** None. **R.D. Andrew:** None. **B.M. Bennett:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.04

Topic: A.07. Developmental Disorders

Support: NIH Grant NS086933-01
NIH AG 064465
NIH T32 MH016880
NIH T32 AG052371
Linda Crnic Institute

Title: Sleep impairment rescue in Dp16 Down syndrome model mice by RCAN1 gene dose correction

Authors: *P. CAIN, H. WONG, C. BORSKI, A. COOPER-SANSONE, L. LAPLANTE, J. LEVENGA, M. OPP, C. HOEFFER;
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Abstract: Sleep impairment rescue in Dp16 Down syndrome model mice by *RCAN1* gene dose correction
Down syndrome (DS) occurs in 1 of 700 to 1000 births and is the most common cause of intellectual disability. DS also results in sleep abnormalities affecting up to 60% of the DS population. Although some sleep disturbances are caused by anatomically derived sleep apnea, many are not. Genes arising from the triplication of human chromosome 21 (Hsa21) may also contribute to sleep disturbances in DS. These abnormalities include increased latency to non-rapid eye movement (NREM) sleep, sleep fragmentation and reduced rapid eye movement (REM) sleep. Among the genes triplicated in DS is the gene for *Regulator of Calcineurin 1* (*Rcan1*, also known as *Down Syndrome Critical Region 1 (DSCR1), calcipressin, modulatory calcineurin interacting protein (MCIP)),* which is chronically overexpressed in the brains of DS individuals and known to impact learning, memory, affective behavior, and synaptic plasticity. Overexpression of RCAN1 can lead to age-dependent cognitive and synaptic impairments. Previously we observed sleep defects in Dp(16)1Yey mice (Dp16) mice (*Levenga* et al. 2018). Because RCAN1 plays a role in circadian function, we hypothesized that increased levels of RCAN1 protein resulting from trisomy Hsa21 may be responsible for sleep disruption in DS individuals. Dp16 are trisomic for ~115 genes orthologous to Hsa21 syntenic regions (Mmu16) while not being confounded by additional genes unrelated to DS. Experimenters blind to genotype examined sleep architecture and EEG patterns in both male and female, young (6-7/group) and aged (7-9/group) WT and Dp16 mice. We also restored disomic levels of RCAN1 in Dp16 mice (Dp16/*Rcan1*^{2N}) and tested sleep and EEG parameters in this RCAN1 dose corrected group (young and aged) to determine if RCAN1 dosage correction could restore normal sleep patterns in Dp16 model mice. Surprisingly, young (2-6 months) Dp16 mice exhibited no sleep architecture or EEG abnormalities relative to wild-type (WT) littermates. However, in aged (10-18 months) mice we found marked sleep and EEG changes compared to WT mice and that RCAN1 dosage correction reduced sleep deficits and partially rescued EEG phenotypes compared to Dp16 mice.

Disclosures: P. Cain: None. **H. Wong:** None. **C. Borski:** None. **A. Cooper-Sansone:** None. **L. LaPlante:** None. **J. Levenga:** None. **M. Opp:** None. **C. Hoeffer:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.05

Topic: A.07. Developmental Disorders

Support: NINDS R01NS105138
NINDS R21HD098542
NINDS F31NS118968

Title: Oligodendrocyte Development is Perturbed in a Region Dependent Manner in Down Syndrome

Authors: *J. A. KLEIN[1], Z. LI[2], S. RAMPAM[3], J. CARDINI[3], E. ZELDICH[4], T. F. HAYDAR[2];
[1]Boston Univ. Grad. Program For Neurosci., Boston, MA; [2]Ctr. Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [3]Biomed. Engin., [4]Anat. and Neurobio., Boston Univ., Boston, MA

Abstract: Down syndrome (DS) is caused by triplication of chromosome 21 (HSA21). It is the most common genetic form of intellectual disability (ID) with a prevalence of 1 in 750 live births. Trisomy 21 results in global transcriptional dysregulation and previous work has shown that one of the alterations is the downregulation of a network of genes regulating the oligodendrocyte (OL) lineage. This suggests that perturbed OL development may be cause of the decrease in white matter in the brains of people with DS that contributes to the ID. To study the mechanism of this deficit, we differentiated isogenic lines of iPSCs derived from people with DS into OLs using small molecules and growth factors to mimic intrinsic development. Additionally, to examine the intersection of spatial patterning and trisomy 21 on OL development, we patterned our cultures to either brain-like or spinal-cord like fate. Using this system, we have identified changes in OL differentiation that are dependent both on genotype and on regional patterning as well as phenotypes that are consistent between regions. One phenotype that is dependent on spatial patterning is the transition of trisomic cells from the neural progenitor cell (NPCs) to pre-OL progenitor cell (pre-OPC) state. This transition is tightly regulated by two transcription factors, OLIG2 and NKX2.2, which are essential to promote OL lineage commitment. In the brain-like pre-OPCs, trisomic cells show a significant increase in OLIG2 expression and a decrease in the expression of NKX2.2. However, in the spinal cord-like condition, there is no difference in expression of OLIG2 or NKX2.2 in trisomic cells. The changes in gene expression in trisomic cells in the spatially patterned cultures is not restricted to changes in OLIG2 and NKX2.2. Using RNA-seq, we found thousands of genes that are dysregulated in trisomic cells between conditions, with a minority of the genes dysregulated in both conditions. These differences emphasize the importance of recognizing the spatial heterogeneity in DS and that different regions may not be dysregulated in the same manner based on their developmental history and trajectory.

Disclosures: J.A. Klein: None. **Z. Li:** None. **S. Rampam:** None. **J. Cardini:** None. **E. Zeldich:** None. **T.F. Haydar:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.06

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD090180
NIH Grant P3016056
Grant from The Children's Guild Foundation.

Title: Coat color-facilitated efficient generation and analysis of the mouse model of Down syndrome triplicated for all human chromosome 21 orthologous regions

Authors: *Y. YU[1], Y. YI[1], Z. XING[1], T. YU[1], A. PAO[1], M. DAADI[2];
[1]Roswell Park Comprehensive Cancer Ctr., Buffalo, NY; [2]Texas Biomed. Res. Inst., San Antonio, TX

Abstract: Down syndrome (DS) is one of the most complex genetic disorders and a leading genetic causeof developmental intellectual disability. The mouse has been an essential modeling organism inDS research because human chromosome 21 (Hsa21) is orthologously conserved with threeregions in the mouse genome. Recent studies have revealed complex interactions amongdifferent triplicated genomic regions and Hsa21 gene orthologs which underlie major DSphenotypes. Since we do not know conclusively which triplicated genes are dispensable in suchinteractions for a specific phenotype, it is desirable that all evolutionarily conserved Hsa21 geneorthologsaretriplicatedinacompletemodel.Forthisreason,Dp(10)1Yey/+;Dp(16)1Yey/+;Dp(17)1Yey/+ mouse is the most complete model of DS to reflectgene dosage effects because it is the only mutant triplicated for all Hsa21 orthologous regions.Recently, several groups have expressed the concern that the efforts needed to generate thetriple compound model would be so overwhelming that it may be im-practical to take advantageof its unique strength. To overcome such an impression, we presented a strategy to drasticallyimprove the efficiency of developing the triple compound model with aid of the targeted coatcolor and confirmed that the mutant generated via such an approach exhibited cognitive deficits.

Disclosures: Y. Yu: None. **Y. Yi:** None. **Z. Xing:** None. **T. Yu:** None. **A. Pao:** None. **M. Daadi:** None.

Poster

P056. Down Syndrome

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P056.07

Topic: A.07. Developmental Disorders

Title: Genetic reduction of eEF2K ameliorates memory deficits and rescues synaptic failure in a mouse model of Down syndrome

Authors: *X. WANG[1], Q. YANG[4], X. ZHOU[2], T. MA[3];
[1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [3]Intrnl. Medicine-Geriatrics, Wake Forest Univ. Sch. of Med., Winston Salem, NC; [4]Geriatrics, Wake Forest Sch. Sch. of Med., Winston-Salem, NC

Abstract: Down syndrome (DS) is one of the commonest genetic diseases. Patients with DS usually develop Alzheimer's disease (AD) in their 40s, which will exacerbate their cognitive dysfunction. Previous studies have shown that synaptic failure is a common character of both AD and DS, while *de novo* protein synthesis is vital for synaptic plasticity, leading to the hypothesis that impaired *de novo* protein synthesis might contribute to the pathogenesis of DS. Eukaryotic elongation factor 2 (eEF2) is a part of the ribosome machinery and facilitates transfer of tRNA from A site to P site, thus plays important role in *de novo* protein synthesis. eEF2 can be phosphorylated and deactivated by the only known kinase, eukaryotic elongation factor 2 kinase (eEF2K), which will impair *de novo* protein synthesis. Here we explored the role of eEF2 in cognitive dysfunction of DS. We first checked whether eEF2 was hyperphosphorylated in the brain of Down syndrome patients and we found that phosphorylation of eEF2 was indeed increased in DS patients compaired to controls. The increase of eEF2 phosphorylation in DS patients was also recapitulated in the Ts65Dn mouse model of DS. To further explore the role of eEF2 in DS, we crossed eEF2K+/- mice to Ts65Dn mice, generating four genotypes: WT, eEF2K+/-, Ts65Dn, and Ts65Dn; eEF2K+/-. We found that memory deficits were ameliorated in Ts65Dn; eEF2K+/- mice compared to Ts65Dn mice as assessed by Novel Object Recognition and Morris Water Maze tests. Electrophysiological study showed that synaptic failure (LTP) in Ts65Dn mice was rescued by knocking down eEF2K. These data suggest that genetic reduction of eEF2K can ameliorate memory deficits and rescue synaptic failure in a mouse model of Down syndrome, which provides a potential pharmaceutical target for the treatment of cognitive dysfunction in DS.

Disclosures: X. Wang: None. **Q. Yang:** None. **X. Zhou:** None. **T. Ma:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.01

Topic: B.07. Synaptic Plasticity

Support: NIH R35 NS111562

Title: Developmental Regulation of Homeostatic Plasticity in Mouse Primary Visual Cortex

Authors: *W. WEN[1], G. TURRIGIANO[2];
[1]Dept. of Biol., [2]Dept of Biol., Brandeis Univ., Waltham, MA

Abstract: Homeostatic plasticity mechanisms maintain both neuronal and network stability by adjusting excitation, inhibition, or the intrinsic excitability of neurons, but the developmental regulation of these distinct forms of homeostatic plasticity remains poorly understood. A major reason behind this information gap is the lack of a uniform paradigm for chronically manipulating activity at different developmental stages. To overcome this limitation, we utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to directly suppress neuronal activity of pyramidal neurons in layer (L) 2/3 of mouse primary visual cortex (V1) at two essential developmental timepoints: the classic visual system critical period (CP, P24-29), and adulthood (P45-55). We show that 24 hours of DREADD-mediated activity suppression simultaneously induces excitatory synaptic scaling up and intrinsic homeostatic plasticity in L2/3 pyramidal neurons during the CP, consistent with previous observations using prolonged visual deprivation. Importantly, DREADD-mediated homeostatic mechanisms show similar molecular characteristics as those induced pharmacologically or via visual deprivation, such as protein interactions involving GluA2 C-terminal tail and Shank3. We next applied the same paradigm to adult animals. Surprisingly, while excitatory synaptic scaling persisted into adulthood, intrinsic homeostatic plasticity was completely absent. Instead, we found that these adult neurons homeostatically adjusted quantal inhibitory inputs, which was absent in their younger counterparts. Thus, the same population of neurons can express distinct sets of homeostatic plasticity mechanisms at different development stages. Our findings suggest that individual homeostatic plasticity mechanisms can be recruited in a modular manner, and can be turned on and off according to the evolving developmental needs.

Disclosures: W. Wen: None. **G. Turrigiano:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.02

Topic: B.07. Synaptic Plasticity

Support: NINDs Grant R35NS097212

Simons Foundation Grant P0529284 to GWD

Title: Rapid homeostatic modulation of synaptic connectivity in the adult mouse hippocampus

Authors: *P. CHIPMAN[1], L. C. PANZERA[2], D. KARMELIC[1], S. J. BERGERSON[2], R. D. FETTER[1], M. B. HOPPA[3], G. W. DAVIS[4];
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Abstract: The ability of any cell to mount an adaptive response and, thereby, counteract a physiological stress is fundamental to basic cell biology. Many of these adaptive mechanisms are at the forefront of efforts to combat disease such as the unfolded protein response and autophagy. Homeostatic plasticity refers to a suite of adaptive physiological mechanisms that allow neurons to respond to disruptions that alter their electro-chemical signaling. In particular, presynaptic homeostatic plasticity (PHP) describes how neurons adjust the release of neurotransmitter to offset disruptions that impair synapse integrity and function. To date, the mechanisms of PHP have been documented, and molecular mechanisms described primarily at neuromuscular synapses in *Drosophila*, mouse and human. Little is known about the process of PHP within the mammalian brain, and the molecular mechanisms remain largely uncharacterized. Now we provide evidence for the rapid induction of PHP at synapses in the CA1 region of adult hippocampus. Partial antagonism of postsynaptic AMPA receptors induces a compensatory increase in presynaptic vesicle release, assessed both electrophysiologically and by direct optical imaging of presynaptic glutamate release. This homeostatic mechanism can be induced within minutes and current evidence argues that the activity of both NMDA receptors and kainate receptors are required. Remarkably, the expression mechanism includes evidence of rapid, *de novo* synapse formation as determined by ultrastructural reconstruction of hippocampal volumes.

Disclosures: P. Chipman: None. **L.C. Panzera:** None. **D. Karmelic:** None. **S.J. Bergerson:** None. **R.D. Fetter:** None. **M.B. Hoppa:** None. **G.W. Davis:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.03

Topic: B.07. Synaptic Plasticity

Support: R01 NS065992

Title: Changes in spiking activity as a trigger for GABAergic versus AMPAergic synaptic scaling in mouse cortical neurons

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Abstract: Neuronal circuits respond to perturbations that challenge their spiking activity by triggering a set of homeostatic plasticity mechanisms, which are thought to restore firing rates. The most widely studied form of homeostatic plasticity is synaptic scaling. Synaptic scaling is characterized by multiplicative changes in the strength of the synaptic inputs onto a neuron, measured as the amplitude of miniature postsynaptic currents (mPSCs). Since the upscaling of excitatory AMPAergic synapses can be produced experimentally by chronic treatments of either a voltage-activated sodium channel blocker (TTX) or AMPA receptor antagonists such as CNQX in neuronal cultures, it had been assumed that excitatory synaptic scaling was triggered by a decrease in spiking activity produced by the AMPAergic blockade. However, we showed previously in cortical cultures that chronic blockade of AMPAergic transmission with CNQX produced the same level of upscaling of AMPA mPSCs whether spiking was reduced or optogenetically maintained at control levels. This suggested that reductions in glutamatergic neurotransmission, rather than spiking activity was the trigger for AMPAergic synaptic scaling. We now have evidence that the mechanisms triggering AMPA- and GABAergic scaling are distinct. Chronic treatments that reduce both spiking and AMPA receptor activation, trigger compensatory GABAergic downscaling. Conversely, chronic treatments that increase spiking and AMPA receptor activation, trigger compensatory GABAergic upscaling. Using mouse cortical cultures we have tested the importance of spiking vs. glutamatergic transmission in triggering GABAergic scaling. We have found that combining treatments that block spiking activity (TTX, triggers GABAergic downscaling) but increase AMPA receptor activation (cyclothiazide, triggers GABAergic upscaling) triggers GABAergic downscaling. This result is consistent with the idea that GABAergic synaptic scaling is triggered by changes in spiking levels and not by changes in AMPAergic transmission. Further, we show that blocking glutamatergic neurotransmission with CNQX while optogenetically maintaining spiking activity at normal levels prevents the GABAergic downscaling that is observed with CNQX treatment alone. These striking results suggest that GABAergic scaling is triggered by alterations in spiking activity and therefore can homeostatically regulate spike frequency. On the other hand, AMPAergic scaling is triggered by altered glutamatergic transmission, not spiking, suggesting that this form of scaling is less well suited for homeostatic regulation of spiking activity.

Disclosures: C. Gonzalez-Islas: None. **P. Bulow:** None. **P.A. Wenner:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.04

Topic: B.07. Synaptic Plasticity

Title: Spatial regulation of coordinated excitatory and inhibitory synaptic plasticity at dendritic synapses

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Abstract: Traditionally, the flexibility of neuronal network has been shown to mainly rely on the potentiation and depression of excitatory synapses. However, recent evidences revealed several forms of inhibitory plasticity, raising the important question of how GABAergic and glutamatergic plasticity are coordinated during neuronal activity. Here, we characterized a non-Hebbian form of inhibitory postsynaptic potentiation (iLTP) induced by postsynaptic depolarizations of principal cells in hippocampal. Interestingly, the same protocols induced depression at glutamatergic synapses (LTD), thus indicating an anti-homeostatic relation between inhibitory and excitatory plasticity. Photolysis of caged glutamate or caged GABA revealed that the aforementioned glutamatergic glutamatergic LTD and GABAergic iLTP are expressed postsynaptically. Subsequently, we investigated how synaptic plasticity induced at individual glutamatergic spines affects the strength of neighboring GABAergic synapses. To this end we induced “single spine LTP” by pairing the postsynaptic depolarization with repetitive glutamate uncaging at individual spines while simultaneously measuring the strength of adjacent dendritic GABAergic synapses by GABA uncaging. Interestingly, we found that, following the delivery of this hebbian-like protocol, GABAergic synapses located within 3 micrometers from a stimulated spine showed depression (iLTD), while GABAergic synapses at distances > 3 micrometers showed iLTP. This spatial dependent reversion of inhibitory plasticity induced by heterosynaptic plasticity from spines was dependent by calcium influx through L-type voltage gated calcium channels and the activation of the protease calpain. Our findings indicate that local changes of calcium concentration can finely coordinate both glutamatergic and GABAergic synaptic plasticity at dendritic level suggesting that the dendritic E/I balance are selectively tuned in spatially restricted dendritic sub-regions.

Disclosures: M. Ruben: None. **T. Ravasenga:** None. **V. Regio:** None. **A.I. Polenghi:** None. **E. Petrini:** None. **A. Barberis:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.05

Topic: B.07. Synaptic Plasticity

Support: R01NS065992
GRANT12982452

Title: Homeostatic plasticity in preganglionic and postganglionic sympathetic neurons

Authors: *A. RATLIFF[1,2], M. HALDER[1,2], S. HOCHMAN[1,2], P. WENNER[1,2];
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Abstract: *Homeostatic plasticity* describes an array of mechanisms that a cell might use in order to maintain a biologically relevant level of output in response to a change in input, and this has been identified throughout the nervous system. Spinal motoneurons (MNs) have been shown to utilize such mechanisms in response to chronic reductions in synaptic input during embryonic development, when the circuit is first functional. Homeostatic mechanisms include compensatory changes in synaptic strength and intrinsic cellular excitability. Regulation of activity in the sympathetic nervous system (SNS) is imperative for healthy organ function and is under tight homeostatic control. However, homeostatic plasticity has been largely ignored in the sympathetic preganglionic neurons (SPNs) and postganglionic neurons (PGNs) of the central and peripheral arms of the SNS. To test the homeostatic capacity of these neurons in embryonic development, we are examining cellular responses to synaptic blockade in the embryonic chick. First, the synapse between SPNs and PGNs was tested for functional connectivity using extracellular stimulation and recording. Consistent with previous data, the synapse was found to be immature at embryonic day 10 (E10), but capable of propagating signals from E12 on. Application of sub-saturating concentrations of the ganglionic nAchR blocker Hexamethonium (Hex), leads to reductions in PGN-evoked spiking in E17 PGNs, which do not appear to homeostatically recover within several hours. E13 embryos were then treated with either vehicle or Hex in vivo for 2 days. Intracellular whole cell patch clamp recordings of PGNs were then performed at E15. At present, Hex-treated neurons did not appear to be more excitable than vehicle-treated neurons. These results suggest that the PGNs - the final output of the SNS - may not homeostatically alter their excitability in response to synaptic blockade at this age. Next, SPNs were inspected for evidence of homeostatic plasticity. Previous data has shown that 2-day *in ovo* treatment from E8-10 with the $GABA_A$ antagonist Gabazine (GBZ) triggers a form of homeostatic plasticity called synaptic scaling. To test whether SPNs experience synaptic scaling we are blocking GABAergic synaptic transmission from E8-10. We are currently conducting experiments using a genetically encoded chloride indicator, Clomeleon, to determine whether GBZ treatment triggers scaling in SPNs, identified by an increase in chloride accumulation. This would be the first data of its kind to indicate that the SNS can respond with mechanisms of homeostatic plasticity to changes in synaptic signaling during embryonic development.

Disclosures: A. Ratliff: None. **M. Halder:** None. **S. Hochman:** None. **P. Wenner:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.06

Topic: B.07. Synaptic Plasticity

Support: R01 NS042595
K00NS113422
F32DA051160-01
American Neuromuscular Foundation Career Development Award

Title: Sustained Depolarization Induces Homeostatic Plasticity in Mouse and Human Sensory Neurons

Authors: *J. S. DEL ROSARIO[1], L. A. MCLLVRIED[1], M. Y. PULLEN[1], A. WANGZHOU[2], R. A. SLIVICKI[1], B. A. COPITS[1], Z. BERTELS[1], A. CHAMESSIAN[1], J.-N. LI[1], A. WIDMAN[1], J. YI[1], T. J. PRICE[2], R. W. GEREAU[1];
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Abstract: Structural and functional remodeling of the nervous system, as result of intrinsic and extrinsic environmental changes, serves as a protective mechanism to assure adequate maintenance of overall network activity in the face of mounting experience-induced changes. These adaptive changes, known as homeostatic plasticity, include both synaptic scaling and regulation of intrinsic neuronal excitability. While there is abundant evidence suggesting that alterations in neuronal excitability of nociceptors contribute to the development of different forms of chronic pain, it is still not known whether nociceptors undergo homeostatic plasticity in the context of persistent excitation, or whether dysregulation of these homeostatic mechanisms contributes to the pathophysiology of chronic pain. Using a combination of pharmacologic and optogenetic approaches, we show that sustained depolarization of nociceptors in dorsal root ganglia (DRG) strongly reduces intrinsic excitability of mouse and human DRG neurons. These changes include decreases in input resistance, action potential (AP) fall time and AP half width, an increase in rheobase associated with an overall decrease in AP firing. This suggests that homeostatic mechanisms are present in mouse and human nociceptors. Surprisingly, RNA sequencing studies of mouse and human DRG neurons did not reveal significant changes in gene expression associated with the induction of this homeostatic plasticity, suggesting the likelihood of potential post-translational modifications as underlying mechanisms. Our data reveal the presence of intrinsic mechanisms of regulation of neuronal excitability in response to sustained depolarization of mouse and human nociceptors. Dysregulation of these homeostatic mechanisms could contribute to the understanding of the pathophysiology of chronic pain syndromes.

Disclosures: J.S. Del Rosario: None. **L.A. Mcllvried:** None. **M.Y. Pullen:** None. **A. Wangzhou:** None. **R.A. Slivicki:** None. **B.A. Copits:** None. **Z. Bertels:** None. **A. Chamessian:** None. **J. Li:** None. **A. Widman:** None. **J. Yi:** None. **T.J. Price:** None. **R.W. Gereau:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.07

Topic: B.07. Synaptic Plasticity

Support: 2R01NS065992-09
T32NS096050

Title: Spike frequency within a burst, rather than overall spike frequency, is homeostatically maintained in the embryonic chick spinal cord

Authors: *A. LAKHANI[1], Z. SABRA[1], N. AU YOUNG[1], P. A. WENNER[2];
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Abstract: Spontaneous network activity (SNA) produces highly synchronized patterns of activity that occur in almost all developing neural networks, including the hippocampus, retina, neocortex, and spinal cord. The episodic bursts are important for numerous functions, such as neurogenesis, neuronal survival, and neurotransmitter expression. Importantly, SNA is crucial for the developing system to shift from a silent to a fully functioning structure with a myriad of synapses and signals. SNA in the spinal cord recruits motoneurons and therefore drives embryonic limb movements, which are crucial for proper muscle and joint development. In the isolated spinal cord of the chick embryo, SNA is expressed as episodes of activity that occur every ~10 minutes and last up to a minute. Each episode is composed of several bursts of depolarizations. Homeostatic plasticity represents a set of mechanisms that ensures the maintenance of activity levels following perturbations and has been well-studied in this system. Previous research from our lab has demonstrated that following in ovo GABAergic blockade, when GABA is excitatory, embryonic movements decrease, but then are homeostatically recovered within 12 hours. However, this is a gross measurement of muscle activity; thus, it is unclear what specifically is maintained in terms of spiking at the cellular level. To address this issue, I isolated embryonic day 11 spinal cords and used a 32-channel NeuroNexus probe to record neuronal spiking during episodes before and after GABAergic blockade. This perturbation, with some variability, reduced episode frequency and number of spikes per episode, which never fully recovered. On the other hand, the average spike frequency within a burst (SFWB) was homeostatically recovered in many motoneurons. Shortly after adding a GABAergic antagonist, there was a dramatic increase in SFWB, but after 1.5-2 hours, SFWB decreased and returned to baseline levels, even though total spikes did not recover. The bursts in the isolated spinal cord correspond to limb movements that occur in the living embryo. These results suggest that the developing system aims to preserve this crucial kicking behavior by monitoring and homeostatically maintaining the SFWB within an episode. By ensuring that motoneurons respond to a synaptic barrage in a consistent manner, the system can appropriately

maintain motor output of the developing cord. We are now examining the correlates of this finding in two different in vivo systems - the chick embryo and the mouse barrel cortex.

Disclosures: A. Lakhani: None. **Z. Sabra:** None. **N. Au Young:** None. **P.A. Wenner:** None.

Poster

P114. Homeostatic Plasticity: Description and Properties

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P114.08

Topic: B.07. Synaptic Plasticity

Support: F31 MH11511
T32 MH19524
NOGLSTP Out to Innovate

Title: Synaptic homeostasis transiently appropriates Hebbian plasticity to elicit an oscillatory response to inactivity

Authors: *S. SUN[1], D. LEVENSTEIN[3], B. LI[4], N. MANDELBERG[5], B. SUUTARI[7], N. CHENOUARD[8], G. TIAN[5], S. SANCHEZ[6], R. W. TSIEN[2];
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Abstract: In neurons, homeostatic plasticity maintains synaptic strength within a functional range through forms of negative feedback.While it is conceptually thought to oppose destabilizing positive-feedback changes of Hebbian plasticity, many molecular components implicated in one are key for the other. Computational models using homeostatic synaptic scaling with Hebbian learning rules fail to maintain stable networks due to temporal discrepancies. These observations raise questions about the current conceptual frameworks of synaptic homeostasis. We discovered that homeostatic synaptic plasticity is a slow damped oscillatory response that links both Hebbian and homeostatic plasticities via L-Type Calcium Channels. Ca^{2+}/CaM-dependent kinases and phosphatases both act on AMPARs to homeostatically regulate synaptic weight by transiently appropriating elements involved in long-term potentiation (LTP) and depression (LTD). We propose a model of synaptic activity that uses pre- and postsynaptic feedback mechanisms that invites a broad reconsideration of Hebbian and homeostatic plasticity as unified, rather than oppositional forces.

Disclosures: S. Sun: None. **D. Levenstein:** None. **B. Li:** None. **N. Mandelberg:** None. **B. Suutari:** None. **N. Chenouard:** None. **G. Tian:** None. **S. Sanchez:** None. **R.W. Tsien:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.01

Topic: B.12. Demyelinating Disorders

Title: Lack of antibody response to SARS-CoV-2 infections and vaccinations in MS patients on anti-CD20 therapy may be independent of CD19 B-cell counts

Authors: J. LIU[1], G. FEUER[1], V. KIRSCHNER[1], J. LEI[1], M. MALIN[1], M. ROCHE[1], *J. LIN[2], R. ALFONSO[1], S. A. SADIQ[3];
[1]Tisch MS Res. Ctr. of New York, New York, NY; [2]Tisch MS Res. Cntr NY, New York, NY; [3]Tisch MS Res. Ctr., New York, NY

Abstract: The COVID-19 pandemic has resulted in an unprecedented accelerated vaccine development against SARS-CoV-2. The vaccines have been shown to effectively induce immune responses in healthy individuals against the virus. However, for multiple sclerosis (MS) patients on anti-CD20 therapy, the ability to generate a protective antibody response may be compromised. At Tisch MS Research Center of New York (MSRCNY), we are exploring the antibody responses of these MS patients after receiving COVID-19 vaccines or after naturally occurring infections. To assess overall IgG antibody response, we used an in-house ELISAs to detect IgG antibodies against the recombinant SARS-CoV-2 S1 subunit's receptor binding domain. To confirm antibody response, samples must show a change in antibody levels from a pre-pandemic or pre-vaccination serum sample. If these prior samples are not available, samples must show positive neutralization activity with a surrogate virus neutralization ELISA. The serum samples used in these assays were obtained from MS patients who have been on anti-CD20 therapies for at least 6 months and were vaccinated and/or naturally infected with COVID at least 1 month prior to blood collection. As part of patients' routine clinical management, CD19 B-cell counts were obtained prior to their next therapy, allowing us to access B-cell counts at the time of serum analysis. Control samples were obtained from non-MS staff members of Tisch MSRCNY at least 1-month post-vaccination or after natural infection. Of 76 vaccinated and/or infected MS patients on anti-CD20 therapy, only 10 had detectable antibody responses. In contrast, all the vaccinated or infected control samples (n=38) that we tested neutralizing antibodies and IgG levels for had detectable antibody responses. These results show decreased antibody response in anti-CD20 treated MS patients. Interestingly, 9 out the 10 MS patients with detectable antibody responses had below normal CD19 B-cell levels, suggesting that the ability to mount a protective antibody response may involve more than just the presence of B-cells.Our results indicate an impaired antibody response to SARS-CoV-2 vaccination and/or natural SARS-CoV-2 infections in MS patients on anti-CD20 therapy. However, the impaired antibody

response may not correlate completely with CD19 B-cell levels. This project is still ongoing as we continue to collect more data to corroborate the findings from this preliminary data.

Disclosures: J. Liu: None. **G. Feuer:** None. **V. Kirschner:** None. **J. Lei:** None. **M. Malin:** None. **M. Roche:** None. **J. Lin:** None. **R. Alfonso:** None. **S.A. Sadiq:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.02

Topic: B.12. Demyelinating Disorders

Support: R01NS104021
RG-1701-26750

Title: Delayed oligodendrocyte progenitor cell recruitment and maturation in large volume demyelination

Authors: *J. J. M. COOPER, J. J. POLANCO, D. SARASWAT, J. J. PEIRICK, F. J. SIM; Univ. at Buffalo-Jacobs Sch. of Med. and Biomed. Sci., Buffalo, NY

Abstract: Remyelination-inducing therapies have the potential to restore neurologic function and prevent progression in multiple sclerosis (MS). However, preclinical therapies capable of driving oligodendrocyte differentiation and enhancing remyelination in murine models have not yet yielded meaningful clinical improvement. These setbacks suggest that small animal models may be inadequate for the development and identification of promising regenerative therapies. Unlike MS, rodent models of demyelination undergo highly efficient remyelination, have typically very small volumes and exhibit abundant oligodendrocyte recruitment rapidly following demyelination. We hypothesized that oligodendrocyte progenitor cell (OPC) recruitment becomes rate-limiting as lesion volume increases leading to a failure of oligodendrocyte differentiation and endogenous remyelination. In this study, we utilized lysolecithin injection into the large white matter tracts of the rabbit to create demyelinated lesions that have cross-sectional areas 20-fold larger than those previously reported in mice, and volumes 8-fold larger. Unlike rodent lesions which show robust supernumerary OPC recruitment (representing a 3-10 fold increase in $Olig2^+$ cell density), recruitment in the rabbit never exceeds normal levels. Importantly, the density of mature $CC1^+$ oligodendrocytes failed to return to NAWM levels by 56 dpl and the percentage of differentiated oligodendrocytes drastically lagged behind that of rodent lesions. These results suggest that as lesion volume increases the requirement for increased OPC proliferation and migration becomes limiting. As oligodendrocyte differentiation from human OPCs is dependent on the local density of progenitors, the reduced density of progenitors in large lesions may contribute to failed

differentiation and remyelination. Together, we show that large volume demyelination in the white matter of the rabbit recapitulates several key features of human MS lesions, and represents an important preclinical model for the assessment of remyelination therapies in human demyelinating disease.

Disclosures: J.J.M. Cooper: None. **J.J. Polanco:** None. **D. Saraswat:** None. **J.J. Peirick:** None. **F.J. Sim:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.03

Topic: B.12. Demyelinating Disorders

Support: Tisch Multiple Sclerosis Research Center of New York (Private funds)

Title: Cerebrospinal fluid antibody analysis of multiple sclerosis patients reveals binding to primarily ubiquitous intracellular antigens

Authors: *G. FEUER, J. LIN, Q. J. YAN, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. of New York, New York, NY

Abstract: Oligoclonal bands (OCBs) are present in over 90% of patients with multiple sclerosis (MS) and indicate the presence of IgG in cerebrospinal fluid (CSF). In order to determine the antigenic target of these IgG, we isolate B cells from the CSF, determine the DNA sequence encoding the variable region, insert this sequence into an expression vector, and produce the antibodies using a human cell expression system. We then utilize the HuProt Human Proteome Microarray produced by CDI Labs. This array consists of over 21,000 GST-purified recombinant proteins grown in Yeast, which allows us to determine antibody specificity on properly folded three-dimensional human proteins. We sent antibodies generated from 22 unique B cells from 15 patients to CDI Labs to be used as probes on the HuProt microarray. All but one of these B cells was clonally expanded. These patients include 8 males and 7 females comprising 5 cases of relapsing-remitting multiple sclerosis (RRMS), 7 cases of primary progressive multiple sclerosis (PPMS), and 3 cases of secondary progressive multiple sclerosis (SPMS). The patients range in age from 24 to 70.
6 of the 22 distinct antibodies sent to CDI Labs had significantly high affinity and specificity for proteins on the array. These 6 antibodies represent 3 RRMS cases, 2 PPMS cases, and 1 SPMS case. Proteins bound by our antibodies include MRPL1 (Mitochondrial Ribosomal Protein L1), MST1 (Macrophage-Stimulating Protein), AKR7A3 (Aldo-Keto Reductase Family 7 Member A3), VCL (Vinculin), HNRNPD (Heterogeneous Nuclear Ribonucleoprotein D), TIA1 (TIA1 Cytotoxic Granule Associated RNA Binding Protein), and MBP (Myelin Basic Protein). They

are all intracellular proteins, except for MST1, which is secreted, and MBP, which is a structural protein in myelin sheaths. 4 out of 7 of these proteins are ubiquitously expressed. We have confirmed binding to TIA1, MRPL1, and MBP through western blotting, which may indicate the presence of a linear epitope that is conserved in the primary structure of the protein upon being denatured. Failure to confirm binding on a western blot for the other proteins may simply be due to differences in binding to the native proteins of the array compared to denatured proteins in the western blot. Binding to MBP has been further confirmed through immunohistology. Our results indicate that antibodies produced in the CSF during disease course may target individualized antigens that vary from patient to patient rather than some antigen common in all cases of MS. Ongoing research seeks to determine the relevance of these particular antigens or if the antigenic targets are truly unique and varied amongst individuals with MS.

Disclosures: G. Feuer: None. **J. Lin:** None. **Q.J. Yan:** None. **S.A. Sadiq:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.04

Topic: B.12. Demyelinating Disorders

Support: National Multiple Sclerosis Society Grant
University of Calgary Biomedical Engineering Undergraduate Award
Alberta MS Network Undergraduate Studentship
Canadian Institutes of Health Research Graduate Funding

Title: Increased light scattering in Multiple Sclerosis measured by frequency-domain near-infrared spectroscopy

Authors: *A. HANSEN[1], D. D. ADINGUPU[1], A. SOROUSH[1,2,3], Q. SHAFQAT[1], T. EVANS[1,2,3], S. JARVIS[3,4], L. BROWN[2], J. F. DUNN[1,2,3];
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Abstract: Multiple sclerosis (MS) is a chronic illness characterized by inflammation, demyelination, and neurodegeneration within the central nervous system. Previously, we utilized frequency-domain near-infrared spectroscopy (fdNIRS) to show that hypoxia is present in the frontal lobe grey matter of about 40% of individuals with multiple sclerosis (MS). The causes of this hypoxia are not known but may relate to a metabolic disorder. It has been proposed that changes in tissue light scattering relate to changes in mitochondrial structure and density and/or to edema. We applied fdNIRS to quantify scattering in three groups: controls, patients with MS

that were normoxic, and patients with MS that were hypoxic. Total oxyhemoglobin and deoxyhemoglobin were quantified with fdNIRS and used to calculate microvessel tissue oxyhemoglobin saturation (S_tO_2) in addition to the reduced scattering coefficient (μ_s) at 690 and 824nm from the frontal cortex of 52 controls and 78 MS patients. S_tO_2 values that were 2 standard deviations below the healthy control mean were defined as hypoxic (<55.7%). Of the individuals with MS, 28 were hypoxic and 50 were normoxic and μ_s was examined between groups with a Kruskal Wallis test followed up with a Dunn's test. The mean ± SD of S_tO_2 was 61.0±5.3 %, 61.9±3.5 % and 49.6±7.1 % in controls, normoxic MS patients and hypoxic MS patients, respectively. Significant differences in μ_s were found between groups when measured at both 690nm and 824nm using the Kruskal Wallis test (p=0.014; p=0.041, respectively). The μ_s at 690nm and 824nm was found to be significantly higher in normoxic MS patients as compared to healthy controls using a Dunn's post-hoc test (10.1±1.3 cm^{-1} vs. 9.3±1.3 cm^{-1}, p=0.009; 8.6±1.3 cm^{-1} vs. 7.9±1.4 cm^{-1}, p=0.017, mean±S.D., respectively). The hypoxic MS population had significantly higher μ_s compared to controls at 690nm (10.0±1.6 cm^{-1}, vs. 9.3±1.3 cm^{-1}, p=0.030) but no detectable difference was found at 824nm (8.2±1.5 cm^{-1}, 7.9±1.4 cm^{-1}, p=0.202). The increased light scattering in MS patients may be a consequence of MS pathology. Previous studies have shown that edema and mitochondrial structure alterations can cause changes in scattering. Mitochondria are dominant scatterers in the near infrared region. Thus, light scattering has been used experimentally to investigate mitochondrial density and volume in literature. These data show that fdNIRS can detect a unique biomarker in patients with MS. This biomarker may relate to mitochondrial dysfunction or edema. fdNIRS may be a useful tool to noninvasively detect underlying pathologies in MS such as cerebral edema, mitochondrial swelling, and hypoxia.

Disclosures: A. Hansen: None. **D.D. Adingupu:** None. **A. Soroush:** None. **Q. Shafqat:** None. **T. Evans:** None. **S. Jarvis:** None. **L. Brown:** None. **J.F. Dunn:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.05

Topic: B.12. Demyelinating Disorders

Support: MGH ECOR Scholars Program
National MS Society Postdoctoral Fellowship

Title: In vivo investigation of histone deacetylase expression in Multiple sclerosis using combined magnetic resonance and positron emission tomography

Authors: *M. C. CATANESE, C.-E. J. TSENG, V. BARLETTA, C. A. TREABA, E. HERRANZ MUELAS, N. R. ZÜRCHER, R. E. G. MARCUS, A. J. PARMAR, B. G.

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Abstract: Multiple sclerosis (MS) is characterized by demyelination and lesion formation in gray and white matter (WM), leading to impaired neurological and cognitive function. MS is a major cause of disability worldwide, with higher prevalence in women compared to men. Currently there is no cure, and the underlying molecular dysregulation is not well understood. Histone deacetylases (HDACs) have been associated with MS, and as such are considered therapeutic targets. Three key lines of evidence link HDAC dysregulation to MS, including altered HDAC expression in postmortem lesion tissue, HDAC mis-localization and interference in axonal transport in both *in vitro* and preclinical models, and multiple studies using several classes of HDAC inhibitors consistently demonstrate symptom amelioration in animal models of demyelination. However, specific alterations to HDAC and their influence on pathology or symptomology in humans remains unknown. Recently, a new window to understanding HDACs in the human brain has opened with [^{11}C]Martinostat, a radiotracer for positron emission tomography (PET). We hypothesize that HDACs will be altered in MS in areas of known pathology and to test this, we measured [^{11}C]Martinostat uptake in six people with relapsing remitting MS (2f/4m;50.3±11.52years) compared to six age- and sex-matched controls (2f/4m;49.16±11.95 years). Dynamic PET images were acquired after bolus injection (controls 5.22±0.41mCi; MS 5.37±0.14mCi) using a combined 3-Tesla:magenetic resonance (MR)-PET system. PET images were reconstructed in units of standard uptake value (SUV), normalized to whole-brain mean (SUVR) and registered to standard space using FreeSurfer and Functional Magnetic Resonance Imaging of the Brain Software Library (FSL). [^{11}C]Martinostat uptake was lower in cortical and subcortical areas in MS compared controls including thalamus, parahippocampus, perirhinal and entorhinal areas and pons (voxelwise analysis using FSL FEAT comparing SUVR between groups; Z>2.3, P-cluster=0.05).
Altered HDAC expression in these areas carry clinical implications. For example, lower HDAC levels in thalamus, an area affected early in the disease course, may lead to altered thalamo-cortical connectivity as sensory inputs relayed from the thalamus influence cortical activation. Future analyses will assess uptake in lesions, normal appearing WM and will evaluate the relationship between HDAC expression and WM microstructure, biological sex as well as neurological function and cognition to establish links between HDAC alterations and symptomology as an important step towards dissecting the involvement of HDACs in MS pathology.

Disclosures: M.C. Catanese: None. **C.J. Tseng:** None. **V. Barletta:** None. **C.A. Treaba:** None. **E. Herranz Muelas:** None. **N.R. Zürcher:** None. **R.E.G. Marcus:** None. **A.J. Parmar:** None. **B.G. Hightower:** None. **A.C. Mehndiratta:** None. **C. Mainero:** None. **J.M. Hooker:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.06

Topic: B.12. Demyelinating Disorders

Support: Adelson Medical Research Foundation

Title: Longitudinal single-nucleus RNA sequencing characterization of an "inside-out" model of multiple sclerosis to elucidate cellular dynamics during myelin loss and repair

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Abstract: Multiple sclerosis (MS), a chronic disease that impacts 2.8 million people worldwide, is characterized by aberrant autoimmune attack against myelin components in the central nervous system (CNS). The loss of myelin-producing cells, oligodendrocytes, is the hallmark of MS; however, the etiology of the initial events is still unclear. Preclinical animal models have been established to investigate different aspects of MS. One such model, the diphtheria toxin A chain (DTA) oligodendrocyte ablation mouse model (*Plp1-CreERT;ROSA26-eGFP-DTA*), involves "inside-out" demyelination, in which autoimmunity against myelin originates from oligodendrocyte death or myelin disruption. After an initial demyelinating phase that peaks 5 weeks post tamoxifen injection (wpi) and resolves 5 weeks later (10 wpi), a secondary phase of demyelination commences about 7 months later. Focal demyelinating lesions form, and neurological disability of suspected autoimmune origins accrues. This model presents an interesting phenotype that parallels the secondary progressive phase of MS. To understand how different types of cells in the CNS participate in oligodendrocyte death and myelin loss, we undertook an in-depth transcriptome characterization of brain cells in white and gray matter of DTA mice across the disease course. Corpus callosum, cortex, and brain stem were examined at symptom onset (3 wpi), at the peak of the initial demyelinating phase (6.3 wpi), during the intermediate recovery phase (17 wpi), and during late-onset secondary demyelination (45 wpi) using single nucleus RNA-sequencing (snRNA-seq). As expected, we observed oligodendrocyte lineage cell (*Mog+* and *Pdgfra+)* loss in both phases of demyelination with temporary reconstitution during the intermediate recovery phase. Neuronal populations (*Slc17a7+* and *Gad1+)* were mostly unaffected, and microglia/immune population elevation (*Ptprc+)* is most prominent at the onset of the first demyelination phase. Interestingly, astrocytic reactivity (*Gfap+)* persists throughout the onset, peak, and recovery phases of demyelination but is attenuated during secondary demyelination, indicating the presence of divergent microenvironments between the two demyelination events. Delineating the differences between these two phases of disease is relevant to understanding the complex pathophysiology of MS.

Disclosures: A. Brake: None. **J. Lin:** None. **R.B. Kunjamma:** None. **Y. Chen:** None. **R. Kawaguchi:** None. **B.J. Popko:** None. **D.S. Reich:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.07

Topic: B.12. Demyelinating Disorders

Title: Vagus Nerve Stimulation (VNS) enhances myelin repair and behavioral recovery following demyelinating injury

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Abstract: Multiple Sclerosis (MS) is an inflammatory, demyelinating disorder that damages oligodendrocytes, resulting in myelin loss and eventual axonal degeneration. While demyelination occurs throughout the central nervous system (CNS), increasing evidence suggests that grey matter demyelination is directly correlated with impaired cognitive and motor dysfunction. Current pharmacological therapies mitigate the frequency of demyelinating events, but do not promote repair of lesions, leading to cumulative damage and functional impairments. There is an unmet need to develop innovative therapeutic approaches that focus on restoration of function through remyelination. Previous studies show that increasing neuronal activity enhances oligodendrocyte generation and myelin plasticity in the healthy CNS. Recent work from our group shows that enhancing neuronal activity via motor learning following demyelination promotes oligodendrocyte regeneration and myelin repair. Vagus nerve stimulation (VNS) drives neuronal activity and plasticity, leading to functional recovery from neuronal injury in stroke, tinnitus and traumatic brain injury. However, whether VNS can be utilized to drive myelin repair and plasticity remains unexplored. We applied chronic VNS to mice with cuprizone-mediated demyelination and used longitudinal two-photon *in vivo* imaging to track the loss and regeneration of oligodendrocytes in motor cortex over time. We found that VNS, when applied in a closed-loop paradigm during dexterous motor learning, enhances remyelinating capacity and results in an increased generation of new oligodendrocytes in motor cortex. To determine if VNS promotes motor function, we evaluated motor performance on a novel dexterous motor learning task. Preliminary data suggests that VNS restores demyelination-induced learning deficits and provides long-term benefits on motor performance. In conclusion, we found that VNS enhances remyelination and the timing of VNS, paired with task execution, may be essential for this improvement. Our results also strongly implicate that VNS accelerates the restoration of deficits

in motor learning and provides long-term improvement in performance following demyelination injury.

Disclosures: R. Huang: None. **E.G. Hughes:** None. **C.G. Welle:** None.

Poster

P177. Human and Animal Studies and Therapeutics: Multiple Sclerosis

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P177.08

Topic: B.12. Demyelinating Disorders

Support: Mayo Clinic Center for MS and Autoimmune Neurology (CMSAN)
The Eugena and Marcia Applebaum Foundation
Mayo Clinic Center for Biomedical Discovery (CBD)
NIH R01NS052741-10
NIA R01AG058812
NMSS G-1508-05951
NMSS RG-1901-33209

Title: Critical role for astrocyte NAD^+ glycohydrolase in myelin injury and regeneration

Authors: *M. R. LANGLEY, C.-I. CHOI, T. R. PECLAT, Y. GUO, W. SIMON, H. YOON, L. KLEPPE, C. F. LUCCHINETTI, C. C. S. CHINI, E. N. CHINI, I. A. SCARISBRICK;
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Abstract: Myelin disturbances and oligodendrocyte loss can leave axons vulnerable leading to permanent neurological deficits. The results of the current study suggest that metabolic disturbances, triggered by consumption of a diet high in fat, cause disruptions in myelinating cells and astrocytes within the mouse CNS. CD38 shows increased expression in the cuprizone and EAE models of demyelination and is the main NAD^+ depleting enzyme in the CNS. Altered NAD^+ metabolism has been linked to both high fat consumption and multiple sclerosis (MS). Here we identify increased CD38 expression in the male mouse spinal cord following chronic high fat diet consumption, after focal toxin (lysolecithin)-mediated demyelinating injury and in reactive astrocytes within active MS lesions. We show that CD38-catalytically inactive mice are significantly protected from high fat-induced NAD^+ depletion, oligodendrocyte loss, oxidative damage, and astrogliosis. Significantly, oral administration of a CD38 inhibitor,78c, increased counts of oligodendrocytes and remyelinated axons after focal lysolecithin-induced spinal cord demyelination. Using a Ribotag approach, we identify a unique *in vivo* brain astrocyte translatome profile induced by 78c-mediated CD38 inhibition in mice, including decreased expression of pro-inflammatory astrocyte markers and increased growth factors. Moreover, in the spinal cords of mice subjected to focal lysolecithin-induced demyelination, the astrocytes had

increased pro-repair markers and reduced pro-inflammatory response markers in the group treated with 78c. Our data suggests that high fat diet impairs oligodendrocyte survival and differentiation through astrocyte-linked mechanisms mediated by the NAD^+ase CD38 and highlight the use of CD38 inhibitors as potential therapeutic candidates to improve myelin regeneration. Together these findings point to a new metabolic targeting strategy positioned to improve disease course in MS and other conditions in which the integrity of myelin is a key concern.

Disclosures: M.R. Langley: None. **C. Choi:** None. **T.R. Peclat:** None. **Y. Guo:** None. **W. Simon:** None. **H. Yoon:** None. **L. Kleppe:** None. **C.F. Lucchinetti:** None. **C.C.S. Chini:** None. **E.N. Chini:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drug containing chow received from Calico Life Sciences, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENC holds a patent on the use of CD38 inhibitors for metabolic diseases.. **I.A. Scarisbrick:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.01

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

Support: R01AI132414

Title: Role of HCMV Infection in Alzheimer’s Disease Pathology

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[1]Cell Biology, Neurobio. and Anat., [2]Microbiology and Immunol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Alzheimer’s Disease (AD) is a common, incurable form of progressive dementia characterized by declining short term memory and reduced mental function. While the mechanisms of AD remain unclear, many modern hypotheses focus on synaptic dysfunction - the earliest pre-clinical manifestation of AD. Previous studies highlight the contributions of environmental pathogens to this process, with Herpes Simplex Type 1 being the archetypal virus. However, other members of Herpesviridae, such as Human Cytomegalovirus (HCMV), are also being interrogated for roles in AD pathogenesis. In animal studies, HCMV infection appears to mirror the AD phenotype via disruption of key synaptic proteins. Further, HCMV has been demonstrated to affect calcium flux and amyloid beta (Aß) accumulation, signaling further overlap with AD. Here, both 3D (cerebral organoid) and 2D (forebrain neuron) cultures will be

used to test whether HCMV infection can influence AD pathology. All cultures utilize induced pluripotent stem cells (iPSCs) derived from either AD (Presenilin-2 mutation) or control (no AD-associated mutations) patients. At day 30 (D30) of differentiation, cultures are either infected with a clinical HCMV strain (TB40/E-GFP variant) or mock treated, with collection at D60. Subsequently, samples will be processed for protein, RT-qPCR, and functional analyses. Preliminary RNA sequencing data of brain organoids reveal HCMV's ability to broadly downregulate gene transcripts, including key synaptic targets affected in AD (Synaptophysin, PSD-95, NMDA/AMPA subunits). If maintained at the protein level, HCMV-mediated loss of these proteins could lead to further synaptic impairment, with potential implications for memory. Further, early experiments show viral modulation of phosphorylated tau (pTau) - the primary component of neurofibrillary tangles (NFTs) in AD - with HCMV driving increases in AD organoids and decreases in control tissues. We hypothesize that HCMV contributes biochemically to the AD phenotype via further downregulation of synaptic proteins and increases in NFT accumulation. In support of this hypothesis, our preliminary electrophysiological results show disproportionate increases in synaptic potentials for AD organoids aligning with hyperexcitability. HCMV infected cells, alternatively, show decreased calcium signaling and synaptic potentials. To date, no studies have investigated potential cooperative effects of HCMV and AD on neuronal function. Together, our current studies will better elucidate HCMV's influence on both the biochemical and functional aspects of AD pathology.

Disclosures: J.W. Adelman: None. **M. Schumacher:** None. **B. O'Brien:** None. **R. Mokry:** None. **S. Terhune:** None. **A. Ebert:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.02

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

Support: CIHR Grant MOP-125901
NSERC Grant RGPIN-05477-2017
Research Manitoba Masters Studentship
Alzheimer Society of Canada Doctoral Fellowship
Alzheimer Society of Manitoba Graduate Studentship
McCrorie-West Family Fellowship for Alzheimer Research

Title: Pannexin1 activation by NMDA receptors is STIM dependent and associated with excitatory synapse deficits caused by amyloid-β oligomers

Authors: *C. S. PATIL, N. E. LAVINE, M. F. JACKSON;
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Abstract: Introduction: During the initial stages of Alzheimer's disease, accumulation of soluble amyloid-β oligomers (AβOs) is associated with loss of Ca^{2+} homeostasis underlying impaired glutamatergic synaptic function. Identifying candidate mechanisms triggering Ca^{2+} dysregulation can thus provide targets for development of novel therapeutics. The focus of our study is to understand synaptic deficits mediated by AβOs through its effect on NMDA receptors (NMDARs) and its downstream signalling effectors, including pannexin1 (Panx1) channels, which have previously been linked to neurological disorders. Overactivation of NMDARs leads to activation of Panx1 is known but we now show that this is regulated by endoplasmic resident stromal interacting molecule proteins (STIMs). Moreover, we show that AβOs sensitizes Panx1 activation, which then promotes synaptic deficits. Methods: Our study utilised two cellular models: Hippocampal neurons cultured from CD1, Panx1 WT and Panx1 KO mice were used for electrophysiology experiments. HEK 293T cells were used for mapping the Panx1-STIM interaction interface. Each result discussed consists of minimum 3 independent experimental replicates. Results: First we show that Panx1 activation by NMDARs was augmented in neurons treated with AβOs. Increased Panx1 activation, coupled with reduced frequency of excitatory synaptic events mediated by AβOs, was observed in WT neurons but not in neurons cultured from Panx1 KO mice. Moreover, Panx1 activation was abrogated in neurons with knockdown of STIMs. Thus, we reasoned modulating STIM-dependent Panx1 activation can serve as a candidate therapeutic target to prevent Aβ mediated synaptic deficits. We created Panx1 deletion mutants and show that STIM activates Panx1 via interaction with a Panx1 N-terminal domain. Moreover, Panx1 activation by NMDARs was abolished in cultured neurons expressing a STIM binding deficient Panx1 mutant. Lastly, using an N-term recognising Panx1 antibody we validated our mechanism of STIM-dependent Panx1 activation. Altogether our results establish the significance of Panx1-STIM interaction in regulating Panx1 channel activation by NMDARs. Future investigations are directed to develop therapeutic targets within our discovered region to inhibit Aβ induced increased Panx1 activity and alleviate synaptic deficits. Conclusion: Our work will help in understanding the role of NMDAR-STIM-Panx1 signalling in mediating physiological and pathological functions in neurons. Further, we hope to provide rationale for developing modality specific therapeutics targeting dysregulated Panx1 function and impeding detrimental effects of AβOs.

Disclosures: C.S. Patil: None. **N.E. Lavine:** None. **M.F. Jackson:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.03

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

Support: NSERC 2020-04617

Title: The effects of amyloid beta protein on excitatory transmission and AMPA receptor phosphorylation in layer II of the entorhinal cortex

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Abstract: In Alzheimer's Disease (AD), neurodegeneration is first found in layer II of the entorhinal cortex, and the degeneration in the EC likely contributes to memory deficits and cognitive impairments that occur early in AD. Current hypotheses regarding the early stages of AD suggest that toxic accumulation of soluble amyloid beta peptides (Aβ) contribute to neurodegeneration. In the hippocampus, elevated Aβ levels interfere with excitatory synaptic transmission by facilitating postsynaptic calcium influx into the neuron via NMDA glutamate receptors, leading to phosphorylation of AMPA glutamate receptors which increase synaptic strength. Chronically elevated calcium levels may contribute to excitotoxicity and eventual cell death in both the EC and hippocampus. Aβ-mediated excitotoxicity and the effects of Aβ on excitatory transmission have been studied extensively in the hippocampus. However much less is known about the effects of Aβ on excitatory synaptic transmission in layer II of the EC. We studied the effects of incubating 400 μm horizontal slices of rat medial EC in 100 nM $A\beta_{(1-42)}$ (n=24) or a dimethylsulfoxide control (n=25) for 45 minutes to 3 hours on excitatory synaptic transmission in layer II EC using field excitatory postsynaptic potentials (fEPSPs) recorded in vitro. We then examined the effects of incubating slices with the addition of 50 μM of D-AP5 to the Aβ incubation (n=13) and the control (n=12). All experiments were blinded. The amplitude of fEPSPs was increased in slices incubated in Aβ relative to control slices. The facilitation of synaptic responses induced by Aβ was blocked by constant bath application of the NMDA receptor blocker D-AP5, indicating that the activation of NMDA receptors is required for the facilitation of synaptic excitability. The facilitation of synaptic responses induced by Aβ likely results from activation of postsynaptic NMDA receptors that enhance calcium influx that can activate CaMKII to result in phosphorylation of AMPA receptors. We assessed tissue samples of the medial EC using the western blotting to measure the expression of CaMKII, AMPA receptors, and phosphorylated AMPA receptors following a one hour incubation in either 100 nM $A\beta_{(1-42)}$ (n=6) or a dimethylsulfoxide control (n=6). We then replicated these experiments with the inclusion of 50 μM of D-AP5 to both incubations (n=6 and n=6). Results of protein analysis were consistent with the electrophysiological recordings. These effects of exposure to Aβ may contribute to mechanisms of excitotoxicity and cognitive decline observed early during the progression of Alzheimer's Disease.

Disclosures: M.E. Suvanto: None. **O.J. Olajide:** None. **J. Saragosa:** None. **C.A. Chapman:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.04

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

Support: AARF-20-685875
RO3 AG060025

Title: Impaired alpha-tubulin re-tyrosination leads to synaptic dysfunction and is a feature of Alzheimer's Disease

Authors: *J. PARATO[1], L. QU[2], A. KUMAR[1], L. PERIS[3], M. PERO[1], A. SPROUL[1], M.-J. MOUTIN[3], A. ANDRIEUX[3], F. BARTOLINI[1];
[1]Columbia Univ. Med. Ctr., New York, NY; [2]Genentech, San Francisco, CA; [3]Grenoble Alpes Univ., Grenoble, France

Abstract: Microtubules in neurons give support to dendrites and axons, provide tracks for transport, and contribute to synaptic plasticity. Dynamic microtubules are composed of tyrosinated tubulin, while a detyrosinated state is associated with increased stability. In the Tyr/deTyr cycle, the C-terminal tyrosine of α-tubulin is removed by tubulin carboxypeptidases and is re-added by tubulin-tyrosine-ligase (TTL). We found that in sporadic Alzheimer's disease (AD), TTL expression is significantly reduced. This finding was recapitulated in a cellular model of familial AD, with human APPV717I London mutation neurons having lower levels of TTL, as well as a decrease in dynamic microtubules. When TTL was knocked down in rat hippocampal neurons, widespread spine loss occurred. Furthermore, we found that spines entered by dynamic microtubules are more resistant to oAβ_{1-42}-induced pruning and that ectopic TTL expression, by promoting microtubule entry into spines, protected the neurons from oAβ_{1-42}-induced spine pruning. Our results demonstrate that tubulin re-tyrosination is decreased in AD, that the Tyr/deTyr cycle contributes to synapse maintenance, and that TTL can prevent oAβ_{1-42}-induced spine pruning.

Disclosures: J. Parato: None. **L. Qu:** None. **A. Kumar:** None. **L. Peris:** None. **M. Pero:** None. **A. Sproul:** None. **M. Moutin:** None. **A. Andrieux:** None. **F. Bartolini:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.05

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

Support: Merit Scholarship from Fonds de recherché du Quebec Nature et technologies

Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Amyloid beta (1-42) peptide-induced impairments in synaptic elements in the entorhinal cortex are associated with perturbed mitochondrial function

Authors: *O. J. OLAJIDE[1], A. BERGDAHL[2], C. A. CHAPMAN[1];
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Abstract: Accumulating evidence suggests that mitochondrial dysfunction and increased reactive oxygen species (ROS) play crucial roles in the synaptic degeneration and neuropathology that drives memory deficits in Alzheimer's disease (AD). We recently showed that solubilized human amyloid beta peptide 1-42 ($hA\beta_{1-42}$) causes rapid alterations at glutamatergic synapses in the entorhinal cortex (EC) through the activation of both GluN2A- and GluN2B-containing subunits of NMDA receptors. Whether the disruption of mitochondrial function contributes to the mechanisms of $hA\beta_{1-42}$-induced synaptic perturbations in the EC is however unknown. The present study focused on assessing the early impacts of $hA\beta_{1-42}$ on EC mitochondrial respiration and the immunoexpression of key components of the mitochondrial respiratory machinery, as well as to determine whether reducing $hA\beta_{1-42}$-induced ROS generation could diminish synaptic dysfunction in the EC. Acute 400 μm horizontal entorhinal slices obtained from 6 to 9-week-old wild-type male long-Evans rats were incubated in artificial cerebrospinal fluid (saturated with 95% oxygen and 5% carbon dioxide) containing exogenous $hA\beta_{1-42}$ (0.5 and 1 μM) or control solution for 3 hours at room temperature. High-resolution respirometry analysis using the Oroboros Oxygraph revealed slight modifications to mitochondrial respiratory measurements in the EC following 0.5 μM $hA\beta_{1-42}$ incubation (n=5), but treatment with 1 μM $hA\beta_{1-42}$ (n=5) caused marked reduction in oxygen consumption relative to control (n=6). $hA\beta_{1-42}$ therefore mediates rapid mitochondrial perturbations in the entorhinal cortex, and this may contribute substantially to early AD-related neuropathology and synaptic degeneration. Changes in the expression of mitochondrial-related proteins and synaptic elements, and the effects of mitochondria-targeted antioxidant compound (Mito-Q) and a synthetic superoxide dismutase and catalase mimetic (EUK 134) on $hA\beta_{1-42}$-treated EC slices will also be discussed.

Disclosures: O.J. Olajide: None. **A. Bergdahl:** None. **C.A. Chapman:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.06

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

Support: Alzheimer's Association Research Fellowship to Promote Diversity (AARF-D) Program 2019-AARFD-643712

Title: Astrocytic tau ablation prevents Ab-induced synaptotoxicity

Authors: *P. CISTERNAS[1], X. TAYLOR[2], O. MALDONADO[1], P. MARTINEZ[1], A. LEE-GOSSELIN[1], C. A. LASAGNA-REEVES[3];
[2]Dept. of Anat. and Cell Biol., [1]Stark Neurosciences Res. Inst. - Indiana Univ., Indianapolis, IN; [3]Stark Neurosciences Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer's disease (AD) is a neurological disorder characterized by the overproduction and accumulation of amyloid-beta (Aβ) and phosphorylation and intraneuronal aggregation of tau. These events promote synaptic dysfunction and loss, which in turn leads to neurodegeneration. Astrocytes, intimately associated with neurons and synapses, are activated by Aβ in a process defined as astrogliosis, emerging as neurotoxic entities. Although astrogliosis has been characterized in AD, the contribution and mechanisms by which reactive astrocytes participate in neurodegeneration remains understudied. Here we show that astrocytic tau is a key factor in the process of synaptic loss. When tau was ablated from astrocytes, the synaptotoxic effects of Aβ-induced astrogliosis were avoided. Interestingly, these tau KO astrocytes showed a neuroprotective phenotype, marked by the upregulated expression of PTX3. Importantly, these results were confirmed by decreasing tau expression on Aβ-stimulated WT astrocytes with a shRNA. Taken together, our results indicate that astrocytic tau is necessary for Aβ-induced astrogliosis to produce synaptic damage. Dissecting the mechanism by which Aβ exerts its neurotoxic effect via tau and identifying new cellular interplays of molecular components in AD will contribute to formulating new strategies for managing this disease.

Disclosures: P. Cisternas: None. **X. Taylor:** None. **O. Maldonado:** None. **P. Martinez:** None. **A. Lee-Gosselin:** None. **C.A. Lasagna-Reeves:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.07

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

Support: NIA P30 AG013854
NIA P30 AG62677
NIA U01 AG06786
NIA K08AG065463
NIA RF1 AG072080
MNP Minnesota Partnership Grant

Title: Phosphorylated tau comparisons in synaptosomal fractions from human brain tissue by sex and APOE genotype

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Abstract: Introduction: Intracellular inclusions comprised of tau protein correlate with, and precede, cognitive deficits in multiple neurodegenerative disorders, such as Alzheimer's disease (AD). The molecular mechanisms by which intracellular tau disrupts synaptic function and impairs cognition, however, remain incompletely elucidated. To address this, we used a highly sensitive, high throughput single molecule array (SIMOA) assay to quantify phosphorylated tau (phospho-tau) and total tau in synaptic fractions and assessed differences when stratified by sex (M/F) and APOE genotype. **Methods**: Frozen tissue was used from entorhinal (ENT) and middle frontal (MF) cortices from 50 age- and sex-matched individuals, 26 of which were female (Northwestern and Mayo Clinic Alzheimer's Disease Centers and Mayo Clinic Study of Aging). Inclusion criteria required a full spectrum of AD Neuropathologic Change (ADNC) severity while excluding significant co-existing lesions. 18 individuals had at least one APOE 4 allele. A fractionation protocol was performed on human brain tissue. Tau was quantified in each separate fraction using Quanterix Simoa-HD1 Platform and Quanterix Tau 2.0 and p-Tau231. Western blotting ensured protein enrichment in appropriate fractions (e.g., PSD95 for P4). Fractional percentages of pTau231 and total tau were calculated. Spearman's Rank-Order correlation was used to detect relationships across ADNC designations. Welch's T-test was used to assess changes in tau by sex and APOE 4 allele status. $P < 0.05$ was considered statistically significant.**Results**: pTau231 ratios of P4 (post-synaptic density) to S4 (pre-synaptic) were initially compared across clinical and neuropathologic designations, demonstrating an increasing trend in the percent of phospho-tau present in P4 fractions of the MF cortex as well as S2 fractions in the ENT cortex with worsening disease. The S4 fraction of the ENT cortex demonstrated an inverse relationship. No significant sex (M/F) differences were identified across groups in S2 ENT cortex ($p=0.9$), S4 ENT cortex ($p=0.7$), or P4 MF cortex ($p=0.9$). When groups were compared with matched levels of tau pathology, those with an APOE 4 allele had decreased pTau231 levels in S4 fraction (e.g., Int. Braak stage cases $p=0.01$).
Conclusions: No, sex specific differences related to total or pTau231 mislocalization were identified across groups. However, the presence of an APOE 4 allele does appear to play a role in the mislocalization of tau in the pathobiologic development of AD. This may be related to neuronal loss with decreased pre-synaptic soluble pTau231. Future studies will focus on clarifying potential underlying mechanisms.

Disclosures: J. Lilek: None. **K. Ajroud:** None. **A. Feldman:** None. **S. Krishnamachari:** None. **T. Gefen:** None. **C. Spencer:** None. **A. Kawles:** None. **G. Minogue:** None. **C. Geula:** None. **P. Jamshidi:** None. **C.R. Jack:** None. **R.J. Vassar:** None. **M. Mesulam:** None. **R.R. Reichard:** None. **M.E. Murray:** None. **D.W. Dickson:** None. **R.C. Petersen:** None. **D. Liao:** None. **M.M. Mielke:** None. **K.M. Nelson:** None. **M.E. Flanagan:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.08

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

Support: U01AG061357
R01AG061800

Title: Defining the neuroprotective mechanisms of neuritin in cognitive resilience to Alzheimer's disease

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Abstract: There is increasing recognition of a subpopulation of “cognitive-resilient” individuals that live to advanced age with intact cognitive function despite the presence of moderate to severe Alzheimer’s disease (AD) pathology. Thus, identifying molecular mechanisms in brain associated with cognitive resilience may reveal novel therapeutic pathways and targets for the treatment and prevention of AD and related dementias. Towards this goal, neuritin (NRN1) was recently identified as the most significant protein associated with cognitive resilience in a proteome-wide association study conducted in the Religious Order Study and Memory and Aging Project longitudinal cohorts (ROSMAP, n=391 individuals), in which higher levels of cortical NRN1 at autopsy correlated with a slower rate of cognitive decline in life. Moreover, in our recent large-scale, multi-center proteomic analysis of >500 human brain tissues, network analysis identified NRN1 as a hub protein in a module enriched with synaptic proteins that positively correlated to global cognitive function and stable cognitive trajectory in life. Collectively, these data support our hypothesis that NRN1 is protective against AD neuropathology and subsequent cognitive decline. To further validate our systems-level data and explore the potential neuroprotective mechanisms of NRN1, we treated rat primary neuronal cultures with recombinant NRN1 protein with or without toxic Amyloid-beta 1-42 (Aβ) oligomer insult followed by dendritic spine morphometric analysis and in-depth proteomics. Morphometric analysis revealed NRN1 treatment in the presence of Aβ oligomers rescued overt

dendritic spine density loss observed with Aβ insult alone. In parallel, multiplex proteomics coupled with off-line fractionation allowed quantification of over 8000 proteins from primary neurons, in which differential and co-expression network-based approaches were applied to identify targets and pathways impacted by NRN1. Notably, modules enriched with proteins involved in biological functions associated with GTPase activity and synaptic transmission were decreased following Aβ insult but rescued when co-incubated with NRN1. Together, our findings support that neuritin protects against amyloid-induced neurotoxicity that may in turn promote cognitive resilience in AD.

Disclosures: C.D. Hurst: None. **D.A. Pugh:** None. **D.M. Duong:** None. **D.A. Bennett:** None. **J.H. Herskowitz:** None. **N.T. Seyfried:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.09

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

Support: AG042178
AG047812
NS105473
AG060767

Title: Protective effects of Mitophagy Enhancers against Amyloid beta-induced Mitochondrial and Synaptic toxicities

Authors: *S. KSHIRSAGAR, H. MORTON, N. SAWANT, A. REDDY, H. REDDY; TTUHSC, Lubbock, TX

Abstract: The aim of our study is to examine the neuroprotective role of mitophagy enhancers such as Nicotinamide, Tomatidine, Urolithin A and Actinonin against Alzheimer’s disease (AD). In recent years, several studies have found reduced fusion and increased mitochondrial fragmentation in a large number of human diseases with mitochondrial dysfunction. Therefore, several groups have found interest in studying mitophagy enhancers. Methods: In our ongoing project, we have examined the protecting role of mitophagy enhancers Nicotinamide, Tomatidine, Urolithin A and Actinonin against impaired defective mitochondrial biogenesis, mitochondrial dynamics, synaptic dysfunction and defective mitophagy in immortalized mouse primary hippocampal cells (HT22) expressing mutant APP (SWI/IND) mutations. From the group of above mentioned mitophagy enhancers, Urolithin A showed strongest effect as compared to others. For our study, we used quantitative RT-PCR, western blot analysis, Seahorse Flux analyzer, biochemical methods and transmission electron microscopy. We

evaluated mutant full-length APP/C-terminal fragments, Aβ levels, mRNA and protein levels of mitochondrial biogenesis, dynamics, mitophagy, and synaptic genes in mAPP-HT22 cells and mAPP-HT22 cells treated with mitophagy enhancers. Results: Increased levels of mitochondrial fission proteins, decreased levels of fusion, biogenesis, mitophagy and synaptic proteins were found in mAPP-HT22 cells relative to HT22 cells. On the other hand, mAPP-HT22 cells treated with mitophagy enhancers compared to mAPP-HT22 cells, we found reduced levels of the mitochondrial fission proteins, enhanced fusion, biogenesis, mitophagy, and synaptic proteins. Our protein data agrees with mRNA levels. Transmission electron microscopy data showed significantly reduced mitochondrial length and increased mitochondrial numbers in mAPP-HT22 cells; these were reversed in mitophagy enhancers treated mAPP-HT22 cells. Cell viability rates were increased in mitophagy enhancers treated mAPP-HT22 relative to mitophagy enhancers-untreated mAPP-HT22. Additionally, seahorse data also showed significantly increased maximal OCR in mAPP-HT22 cells treated with mitophagy enhancers relative to untreated mAPP-HT22 cells, indicating that all mitophagy enhancers showed increased maximal OCR, however, Urolithin A maximal respiration is the highest. Conclusions: These findings suggest that mitophagy enhancers reduces mutant APP and Aβ and mitochondrial toxicities and may have a protective role of mutant APP and Aβ-induced injuries in patients with AD.

Disclosures: S. Kshirsagar: None. **H. Morton:** None. **N. Sawant:** None. **A. Reddy:** None. **H. Reddy:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.10

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

Support: UH Foundation

Title: The Neuroprotective Actions of an Active Core Sequence of Amyloid Beta on Amyloid Beta-Induced Synaptic Dysfunction

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Abstract: Alzheimer’s disease (AD) is a devastating neurodegenerative disorder characterized by cognitive decline and progressive memory loss. Pathological aggregation of soluble beta-amyloid (Aβ) into insoluble Aβ-plaques is one of two prominent histopathological hallmarks of the disease, the other being hyperphosphorylated tau in the form of neurofibrillary tangles (NFTs). Aβ is produced through sequential enzymatic cleavage of the amyloid precursor protein (APP) resulting in a series of Aβ peptides. At physiological levels (pM-nM), we and others have

shown that soluble Aβ acts as a neuromodulator enhancing synaptic plasticity. However, at pathological levels (μM), the accumulation of the $A\beta_{1-42}$ ($A\beta_{42}$) peptide contributes to the significant neuronal cell death and synaptic transmission impairments. Our laboratory has shown that an endogenously cleaved N-terminal Aβ fragment (N-$A\beta_{1-15,16}$) and an essential core sequence, YEVHHQ (N-Aβcore) within the fragment, is neuroprotective against $A\beta_{42}$-induced oxidative stress and apoptosis in *in vitro* and ex *vivo* studies. In an APP/PS1 mouse model, the N-Aβcore injected bilaterally into the hippocampus rescued spatial memory deficits. Furthermore, we have recently reported that the N-Aβcore reversed the impairment in synaptic plasticity in hippocampal slices for both long-term potentiation (LTP) and long-term depression (LTD) in a PI3K/mTOR-dependent manner. However, the molecular mechanisms of the regulatory actions of the N-Aβcore at the synaptic level have yet to be uncovered. Here, we have further investigated the neuroprotective actions of the N-Aβcore on $A\beta_{42}$-induced synaptic dysfunction. To examine at which stage in LTP the N-Aβcore is most effective, acute hippocampal slices were subjected to applications of the N-Aβcore in the presence of exogenous $A\beta_{42}$ for wild-type slices or endogenous $A\beta_{42}$ for slices from the 5xFAD APP/PS1 mouse model at various times across the extracellular recording period. Immunohistochemistry and immunoblots of protein lysates were assessed in parallel for differential expression of AMPAR, NMDARs, and mGluRs at the different time points. To delineate and confirm the involvement of PI3K/mTOR signaling pathways, primary hippocampal neuronal cultures and organotypic slice cultures were treated with $A\beta_{42}$ without or with the N-Aβcore. Protein expression of downstream and upstream signaling of PI3K/mTOR were compared. Elucidating the neuroprotective molecular mechanisms of the N-Aβcore at the synaptic level will provide essential guidance for future use as a potential therapeutic.
Funding: UH Foundation

Disclosures: R.M. Taketa: None. **R.A. Nichols:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.11

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

Support: Training in Pathobiology and Rehabilitation Medicine 2T32HD071866-06 to CMH
R01 AG066489 to LLM

Title: Altered temporal discounting, reduced motivation, and impaired executive functions in the TgF344AD rat model of Alzheimer’s Disease during young adulthood

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Abstract: The relative weight that individuals give to rewards and "costs" (such as delay to reward delivery) in making decisions varies significantly across the population and disease states. One aspect of decision making involves weighing the relative benefits and costs associated with immediate versus delayed outcomes. This aspect of decision making is often referred to as temporal discounting (or intertemporal choice) and can be assessed on tasks in which subjects are required to choose between small, immediate rewards and larger rewards delivered after varying delays. Furthermore, intact executive functioning and motivation are foundational to the decision-making process. Recent literature shows there is a great deal of variability in the temporal discounting phenotype between individuals with mild cognitive impairment, frontal temporal dementia, and Alzheimer's Disease (AD), with some showing no differences relative to healthy controls and others showing altered rates of temporal discounting. Even beyond these disorders, individual differences in choice behavior (either maladaptive or normative) in young adults predict a variety of life outcomes, including educational success and socioeconomic status. The current study used a behavioral approach to determine the effect of genotype on phenotypic differences in temporal discounting, motivation, and executive function in the TgF344AD (TgAD) rat model of AD.
Young adult (6-7 mo) wild-type (WT; n=6) and TgAD (n=6) were trained on several operant tasks including temporal discounting (decision making), progressive ratio (motivation), set shifting (cognitive flexibility), and delayed response (working memory) tasks. Results suggest TgAD rats showed a greater preference for the large, delayed reward relative to WT, however, this preference did not translate to a greater number of total rewards earned. Additionally, TgAD rats were less motivated to obtain the larger reward, were less cognitively flexible, and showed impaired working memory relative to WT controls. Interestingly, young TgAD rats show a similar decision-making and cognitive phenotype as old rats and these data suggest AD pathology may manifest as maladaptive decision making in addition to impaired executive functions early in life.

Disclosures: C.M. Hernandez: None. **N.L. Jackson:** None. **L.L. McMahon:** None.

Poster

P207. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: In Vitro Models I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P207.12

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

Support: NIH R56 AG055497

Title: Lysosome-autophagosome defects mediate proteinopathy in Alzheimer's disease pathogenesis

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by abnormal protein aggregates, intracellular Ca^{2+} dysregulation and memory loss. The catabolic degradation of misfolded protein aggregates, or autophagy, is primarily regulated by lysosomes which require an acidic intralysosomal pH, maintained by the H^{+}-ATPase (V-ATPase). In AD, V-ATPase defects can lead to abnormal β-amyloid and p-tau accumulation. A concurrent mechanism with V-ATPase deficits is intracellular Ca^{2+} dyshomeostasis, which disrupts ion exchange resulting in alkalinization of pH within lysosomes. We hypothesize that Ca^{2+} dyshomeostasis disrupts V-ATPase ion exchange and instigates AD pathology. Here, we performed immunoassays and live cell imaging in model cells, AD mouse models, and induced human neurons (iNs) derived from AD patients to explore this hypothesis. Immunohistochemistry in fixed hippocampal slices from 3-month old 3xTg-AD mice revealed diminished expression of V-ATPase subunits (V1B2, V0a1), lysosomes (Lamp1), and increased expression of mature autophagosomes (LC3B), and hyperphosphorylated tau (p-tau; S262) relative to non-transgenic (NTg) controls. These phenotypes in 3xTg-AD mice were restored to NTg levels by normalizing calcium homeostasis via 30-day treatment with the RyR modulator, Ryanodex (10mg/kg). Similarly, autophagosomes accumulated in iNs derived from AD patients were restored to control levels with Ryanodex treatment. The decreased V-ATPase expression and increased autophagosomes reflect impaired lysosomal function, which is mediated through upstream RyR-Ca^{2+} dyshomeostasis. When measuring lysosomal pH (Lysosensor Y/B Dextran) on iNs and RyR-overexpressing HEK293 cells, it revealed lysosomal alkalization upon RyR stimulation (caffeine 10mM). Ryanodex pre-treatment attenuated this alkalization, suggesting that RyR-Ca^{2+} signaling regulates lysosomal pH and disrupts autophagosome-lysosome mediated protein degradation. Protein aggregates, such as β-amyloid and hyperphosphorylated tau, were increased in iNs with V-ATPase inhibition (bafilomycin 125 nM), which was resolved with Ryanodex treatment. Prior to overt histopathology or cognitive deficits, abnormal RyR-mediated Ca^{2+} release disrupts lysosomal function leading to altered autophagic-mediated protein clearance that contributes to AD proteinopathy.

Disclosures: S. Mustaly: None. **A. Gilman-Sachs:** None. **R.A. Marr:** None. **D.A. Peterson:** None. **K.D. Beaman:** None. **G.E. Stutzmann:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.01

Topic: C.06. Neuromuscular Diseases

Support: LiveLikeLou at the Pittsburgh Foundation
NIH R01NS105756
NIH R21AG064940
Target ALS Foundation
Association for Frontotemporal Degeneration

Title: Functional analysis of GWAS candidate gene, SCYL3, and its role in TDP-43 function and regulation

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Abstract: TDP-43 (transactive response DNA-binding protein of 43 kDa) is a DNA/RNA binding protein involved in RNA alternative splicing, metabolism, and trafficking. TDP-43 is mislocalized from the nucleus to the cytoplasm where it forms pathological insoluble inclusions formed through aberrant liquid-liquid phase separation (LLPS) in a number of neurodegenerative diseases including Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal Lobar Degeneration (FTLD-TDP). A recent genome-wide association study (GWAS) investigating a cohort ranging from moderate to severe AD cases identified several candidate markers associated with increased severity in TDP-43 pathology. SCY1 Like Pseudokinase 3 (SCYL3) encodes a protein with a kinase domain and four HEAT repeats, was implicated as a high potential gene candidate. SCYL proteins are thought to regulate cellular processes such as intracellular trafficking and cell migration; however, the role of SCYL3 in neurodegenerative diseases and TDP-43 pathology remains unclear. To investigate how SCYL3 might contribute to TDP-43 pathology, we performed functional studies in HEK293 cells and iPSC-derived neurons to characterize SCYL3's role in TDP-43 regulation and function. We also examined how SCYL3 affects intracellular aberrant LLPS using a novel optoTDP stable cell line. Our results suggest that SCYL3 may modify TDP-43 cytoplasmic inclusions and is a risk factor for TDP-43 pathology. Taken together, this work integrates data from a GWAS study and functional cell biology analysis to validate a potential gene candidate.

Disclosures: M. Ngo: None. **J.C. Mauna:** None. **J.K. Kofler:** None. **C.J. Donnelly:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.02

Topic: C.06. Neuromuscular Diseases

Support: LiveLikeLou at the Pittsburgh Foundation
NIH Grant R01NS105756
NIH Grant R21AG064940
Association for Frontotemporal Degeneration
Target ALS Foundation

Title: Molecular and cellular pathobiology underlying ALS/FTD causing TDP-43 G295R mutation

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Abstract: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal lobar Degeneration (FTLD) are highly related fatal neurological disorders that manifest by the progressive degeneration of neurons in the motor cortex and spinal cord or frontal and temporal lobes, respectively. TAR DNA-binding protein 43 (TDP-43) is a predominantly nuclear protein involved in splicing and RNA processing that forms cytoplasmic and insoluble inclusions in ~97% of all ALS cases and ~50% of frontotemporal lobar degeneration (FTLD) cases. The study of TDP-43 pathobiology in endogenous model systems remains challenging and, to date, most induced pluripotential stem cell-derived (iPSC) neurons from ALS/FTD patients do not recapitulate significant TDP-43 pathobiology. In this study, we generated iPSC neurons derived from fibroblasts of siblings harboring a c.883GtoC heterozygous missense mutation variant in the TARDBP gene, which results in a TDP-43 p.Gly295Arg substitution. Despite being carriers of the same TDP-43 G295R missense mutation, each sibling presented with different ALS phenotypes: one had spinal onset without ever exhibiting cognitive dysfunction and the other presented with cognitive and behavioral symptoms of FTLD. Notably, TDP-43 G295R iPSC neurons exhibited abnormal TDP-43 staining showing changes in nuclear, cytosolic, and axonal TDP-43 compared to healthy controls suggestive of dysfunctional TDP-43. In this study, we characterize TDP-43 in TDP-43 G295R iPSC neuron lines and examine how this mutation alters TDP-43 function. Specifically, we examined the effect of this mutation on TDP-43 localization/trafficking and autoregulation. Stress granules (SGs) are dynamic membraneless organelles that recruit TDP-43 and other RNA binding proteins and are formed through liquid-liquid phase separation (LLPS). These structures form during periods of cell stress and function to inhibit non-essential protein synthesis until the stress is resolved. Recent work from us and others indicate that pathological TDP-43 liquid-to-solid transitions are driven through aberrant LLPS, thus impaired stress granule dynamics may act as a driver of TDP-43 deposition. Thus, we tested how endogenous TDP-43 G295R affects SG dynamics and whether these are sufficient to induce endogenous TDP-43 inclusions. Taken

together, these data provide insight into the consequence of ALS/FTD causing TDP-43 G295R mutations and highlight a novel iPSC model system of endogenous TDP-43 dysfunction.

Disclosures: J.C. Mauna: None. **A.M. Gleixner:** None. **H.L. Crable:** None. **O.R. Shapiro:** None. **D. Lacomis:** None. **C.J. Donnelly:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.03

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS105756
NIH Grant R21AG064940

Title: The study of Circular RNAs and their regulation of TAR DNA binding protein 43 (TDP-43) pathological aggregation

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Abstract: TAR DNA binding protein 43 (TDP-43) is a nuclear RNA binding protein (RBP) that is found mislocalized to the cytoplasm and forms insoluble inclusions in the CNS of Amyotrophic Lateral Sclerosis (ALS) patients. Functional TDP-43 exists as a soluble protein that condenses to form dynamic liquid-like droplets and localizes to membraneless organelles through liquid-liquid phase separation (LLPS) under physiological conditions. However, recent work indicates that abnormal LLPS, hypothesized to be driven by genetic or environmental factors, initiates the deposition of pathological TDP-43 aggregates observed in neurodegenerative disorders. Work from our lab and others indicates that TDP-43's RNA binding status contributes to the ability of purified and cellular TDP-43 protein to undergo aberrant LLPS and RNA engagement to TDP-43 prevents its pathological condensation. Circular RNAs (circRNAs) are covalently closed circular RNA previously thought of splicing by-products of longer mRNAs transcripts. Recent evidence indicates circRNAs are highly enriched in brain and play a role in neurodegenerative diseases like Alzheimer′s disease (AD), Parkinson's disease (PD) and ALS. While the specific role of circRNAs in neurodegenerative disease pathogenesis remains unknown, a possible explanation is that circRNAs bind to RBPs (i.e. TDP-43) as cytoplasmic molecular sponges to prevent their aberrant LLPS and pathological aggregation. Thus, we hypothesize that circRNA binding to TDP-43 contributes to the maintenance of normal LLPS and prevents the transition to of insoluble and pathological TDP-43 depositions observed in disease. To test this, we first performed in depth RNA sequencing

analyses for circRNAs in postmortem brain samples of neurodegenerative disease patients with and without TDP-43 pathology to identify differentially regulated circRNA species in regions of degeneration. We also performed functional cellular assays using circRNA-expression system to determine how specific RNA species affect the material properties and function of TDP-43. This includes the optoTDP43 cell model that allow for the induction of aberrant LLPS and TDP-43 deposition upon blue light stimulation. This work provides foundational understanding of circRNA:RBPs regulation, which could provide new insight into neurodegenerative disease pathogenesis.

Disclosures: J. Xu: None. **W.M. Tennant:** None. **J.K. Kofler:** None. **C.J. Donnelly:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.04

Topic: C.06. Neuromuscular Diseases

Support: LiveLikeLou at the Pittsburgh Foundation
National Institutes of Health R01NS105756
National Institutes of Health R21AG064940
National Institutes of Health R21NS098379
Target ALS Foundation
Association for Frontotemporal Degeneration

Title: Impact of FG nucleoporin localization irregularities in C9ORF72 ALS and FTLD

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Abstract: Amyotrophic lateral sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) share clinical, neuropathological, and genetic features. A G4C2 repeat expansion mutation in the *C9orf72* gene (C9-ALS/FTLD) is one genetic cause of disease onset. Furthermore, these diseases exhibit cytoplasmic and insoluble protein depositions of the TDP-43 occur in areas of neurodegeneration. Proposed mechanisms of toxicity in C9-ALS/FTLD are the production of

repeat expansion transcripts and their dipeptide repeat proteins (DPRs) products which are hypothesized to drive nucleocytoplasmic transport defects. Nucleocytoplasmic trafficking is facilitated, in part, by the nuclear pore complex (NPC) that contains a selectivity and permeability barrier comprised of phenylalanine glycine nucleoporins (FG nups). However, the relationship between FG nups and TDP-43 pathology remains elusive. Here, we show Nup62 nuclear depletion and cytoplasmic localization in C9-ALS/FTLD and sALS/FTLD and this effect is promoted through interactions with TDP-43. In C9-ALS/FTLD, poly-GR initiates the formation of TDP-43 containing stress granules (SGs) aligning with the nuclear loss and recruitment of Nup62 *in vitro* and *in vivo*. Absent of poly-GR, we find aberrant TDP-43 to sequester Nup62 into pathological inclusions both in cellular systems and postmortem tissue. These changes in nucleoporin localization also show broad implications on a variety of neuronal functions.

Disclosures: A. Gleixner: None. **B.M. Verdone:** None. **C. Otte:** None. **N. Ramesh:** None. **J.C. Mauna:** None. **J.R. Mann:** None. **E.L. Daley:** None. **J. Ortega:** None. **M. Cicardi:** None. **E. Kiskinis:** None. **J.K. Kofler:** None. **U.B. Pandey:** None. **D. Trotti:** None. **C.J. Donnelly:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.05

Topic: C.06. Neuromuscular Diseases

Support: NINDS K081-K08_NS094744-02

Title: Sequence determinants of TDP-43 partitioning into neuronal RNP granules: Effects of ALS linked TDP-43 mutations on neuronal RNP granule properties, function and pathology.

Authors: *S. S. VISHAL, P. P. GOPAL;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a disease of motor neuron degeneration with pathologic inclusions composed of TDP-43, an RNA/DNA-binding protein of 43 kDa. Moreover, mutations in *TARDBP*, the gene that encodes TDP-43, have been linked to both familial and sporadic ALS. TDP-43 regulates critical cellular functions like transcription, RNA splicing, mRNA translation and transport. We and others have shown that TDP-43 undergoes liquid-liquid phase separation and that neuronal TDP-43 ribonucleoprotein (RNP) granules display dynamic liquid-like biophysical properties. TDP-43 contains two RNA recognition motifs (RRM1 and RRM2) flanked by N-terminal domain containing NLS and a C-terminal domain with G/Q/N-rich low complexity domain (LCD). LCD is necessary for interaction with

different proteins and contains an alpha-helical region important for liquid-liquid phase separation. Although most of the ALS-linked mutations in *TARDBP* occur within the LCD of TDP-43, these mutants were reported to have different effects on phase separation in vitro and show distinct biochemical properties, i.e. increased protein half-life vs increased propensity for aggregation vs increased cytoplasmic localization. We asked whether different TDP-43 ALS-linked mutations, LCD mutations and NLS mutation, affect the biophysical properties, axonal transport and recruitment into neuronal RNP granules differently. To test this, we expressed eGFP tagged wild type (WT) TDP-43 or eGFP tagged TDP-43 mutants in rat primary cortical neurons and used live cell confocal microscopy to study TDP-43 WT and mutant RNP granule properties. Kymographs and a custom automated tracking software that measures the anterograde and retrograde flux, velocity, displacement, and run length etc. of each track were used to analyze the motility of RNP granules containing WT or mutant TDP-43. Our results suggest that all the ALS-linked TDP-43 mutations used in this study severely impaired motility of RNP granules. Interestingly, ALS-linked TDP-43 mutations adjacent to the alpha-helical region show substantial impact on long-range transport of TDP-43 RNP granules, reduce anterograde flux and may inhibit partitioning into neuronal RNP granules. Additional preliminary findings suggest that residues within the NLS may be important for intracellular phase separation into neuronal RNP granules. We will now use these ALS-linked TDP-43 mutations and additional LCD mutations (aromatics, alpha helical domain) to determine the contributions of specific residues to intracellular phase separation of TDP-43, how these defects affect critical functions of TDP-43 and promote ALS disease progression.

Disclosures: S.S. Vishal: None. **P.P. Gopal:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.06

Topic: C.06. Neuromuscular Diseases

Support: SB PhD fellowship of FWO-Vlaanderen 1145619N
FWO-Vlaanderen research project grant G0C1620N
KU Leuven (C1 Fund)
ALS association (ALS) 457
ALS Liga België (A Cure for ALS)

Title: Hnrnpk modifies rna toxicity in c9orf72 als through modulation of dna damage response

Authors: *E. BRAEMS[1,2], V. BERCIER[1,2], E. VAN SCHOOR[1,2], J. BECKERS[1,2], L. DEDEENE[1,2,3,5], M. MOISSE[1,2], K. HEEREN[1,2], D. R. THAL[4,6], P. VAN DAMME[1,2,7], B. SWINNEN[1,2,7], L. VAN DEN BOSCH[1,2];

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Abstract: The 'GGGGCC' repeat expansion in *C9orf72* is the most frequent genetic cause of ALS. Three disease-causing mechanisms have been proposed, including a loss-of-function of C9orf72 and two gain-of-functions resulting from either sequestration of essential repeat RNA-binding proteins (RNA toxicity) or from the formation of toxic dipeptide repeat proteins (DPR toxicity). In this study, a C9orf72 ALS zebrafish model was used to identify and investigate modifiers of RNA toxicity. This unique *in vivo C9orf72* RNA toxicity model is characterized by ALS-related motor neuron deficits, including shortened axonal length and aberrant branching in 30 hours post fertilization (hpf) zebrafish embryos. In our modifier screen, mRNA encoding candidate RNA-binding proteins was co-injected with RNA containing 90 repeats of 'GGGGCC' in 1- to 2-cell stage zebrafish oocytes. One of these RNA-binding proteins, hnRNPK, was able to successfully rescue the *C9orf72* repeat RNA-induced axonal phenotype in zebrafish embryos. Functional analysis of the RNA-binding protein hnRNPK uncovered two types of structural domains being essential for modifying RNA toxicity: the nuclear localization signals and RNA-binding domains. These findings suggest that both subcellular localization and RNA recognition play a role in the observed effect. Combined with a distinct cytoplasmic mislocalization of hnRNPK in C9orf72 ALS patient fibroblasts, iPSC-derived motor neurons and post-mortem central cortex, our data hint towards a disrupted hnRNPK function in C9orf72 ALS. Interestingly, using zebrafish bulk RNAseq analysis and CLIPseq datasets we discovered a DNA damage response-related transcriptional target of hnRNPK being involved in the C9orf72 RNA toxicity mechanism. Dysregulation of this downstream effector was also observed in C9orf72 ALS patient post-mortem central cortex. Moreover, siRNA-mediated knockdown of hnRNPK in HeLa cells, confirmed the link between hnRNPK and its target, revealing DNA damage as a novel lead in the understanding of C9orf72 ALS.

Disclosures: E. Braems: None. **V. Bercier:** None. **E. Van Schoor:** None. **J. Beckers:** None. **L. Dedeene:** None. **M. Moisse:** None. **K. Heeren:** None. **D.R. Thal:** None. **P. Van Damme:** None. **B. Swinnen:** None. **L. Van Den Bosch:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.07

Topic: C.06. Neuromuscular Diseases

Title: Poly(adp-ribose) promotes the condensation of c9orf72 arginine-rich dipeptide repeat proteins

Authors: J. GAO, R. PUNATHIL, T. GENDRON, ***K. ZHANG**;
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Abstract: Arginine-rich dipeptide repeat proteins (R-DPRs) is critical to the pathogenesis of *C9ORF72*-mediated amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the most common familial ALS and FTD (c9ALS/FTD). As a pathological hallmark of c9ALS/FTD, R-DPRs aggregate, and sometimes co-aggregate with their interacting proteins, in patient postmortem tissues and animal models. However, what promotes R-DPR aggregation or co-aggregation with other proteins is unclear. Here, we show that poly(ADP-ribose) (PAR) interacts with R-DPRs, promoting their condensation and co-condensation or co-aggregation with their interacting proteins. Loss of PAR polymerase 1 (PARP1) function suppresses PAR levels, R-DPR aggregation, and neurodegeneration in cellular or animal models of c9ALS/FTD. Furthermore, PAR levels are increased in c9ALS/FTD patient postmortem brains. Our findings identified PAR as a contributor to R-DPR toxicity in c9ALS/FTD pathogenesis, suggesting it as a potential therapeutic target and biomarker.

Disclosures: J. Gao: None. **R. Punathil:** None. **T. Gendron:** None. **K. Zhang:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.08

Topic: C.06. Neuromuscular Diseases

Title: C9orf72 associated Dipeptide Oligomers in ALS and FTD

Authors: *N. BHATT, N. PUANGMALAI, M. MONTALBANO, U. SENGUPTA, C. JEREZ, M. KIDD, R. KAYED;
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Abstract: The hexanucleotide repeat expansion in *C9orf72* is one of the most common causes of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The hexanucleotide expansion is a structural polymorphism formed by a GGGGCC repeat region that impedes traditional transcription, and leads to several error-prone transcripts. These transcripts can undergo non-AUG dependent translations, producing several dipeptide repeat-containing proteins (DPRs) including GP, GA, GR, PA and PR. The DPRs form toxic neuronal inclusions in patients with ALS and FTD as suggested by several studies. Some dipeptide repeats such as GA, aggregate as well as sequester other cellular proteins. Given increasing evidence that soluble

species, like oligomers, are the main cause of neurotoxicity in neurodegenerative conditions such as Alzheimer's and Parkinson's disease, we investigated the ability of DPRs to aggregate and form toxic oligomers. Here, we have taken a reductionist approach by synthesizing short dipeptides repeats (GA, PR, and GR) of varying lengths and used biophysical as well as biochemical assays to characterize the aggregates *in vitro*. The results suggest the propensity for DPR, specially GR and PR to form oligomeric structures which can cause toxicity. We also tested the toxicity of the varying DPR aggregates alone and in the presence with tau protein *in vivo*. Moreover, we investigated the presence of DPR oligomers in ALS and FTD human samples and their potential role in disease pathogenesis. Thus, the ability to detect and characterize oligomeric DPR has great potential to further the understanding of these diseases and aid in the development of targeted therapeutics.

Disclosures: N. Bhatt: None. **N. Puangmalai:** None. **M. Montalbano:** None. **U. Sengupta:** None. **C. Jerez:** None. **M. Kidd:** None. **R. Kayed:** None.

Poster

P286. Motor Neuron Disease TDP43 and C9orf72

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P286.09

Topic: C.06. Neuromuscular Diseases

Title: The role of miRNAs on TDP-43 proteopathic seeding

Authors: *J. ZHU[1], S. CHEN[2], M. JACKREL[2], C. WEIHL[1];
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Abstract: TDP-43 is a key component of protein inclusions in 90% of ALS and 50% of FTD patients. In addition, more than 45 different TDP-43 mutations have been identified, accounting for 5-10% of fALS. Similar to other genetic causes of ALS-FTD, TDP-43 belongs to the hnRNP family and functions in RNA metabolism. TDP-43 contains a highly disordered prion-like domain. Recent studies support that TDP-43 can seed and propagate *in vitro* and *in vivo*. We developed a TDP-43 seeding assay using hippocampal neurons, which phenocopied pathologic TDP-43 inclusions. Using this assay, we performed miRNA microarray on TDP-43 seeded vs monomer treated neurons, and identified 8 miRNAs that were significantly changed in TDP-43 seeded neurons but not in controls. Our subsequent studies focused on miR-34a, miR-127, and miR-381, which are conserved across species. Notably, TDP-43 seeding activities were suppressed with overexpression of these miRNAs. Our study supports an effect of selected miRNAs on TDP-43 seeding and enlightens potential targets for ALS-FTD therapy.

Disclosures: J. Zhu: None. **S. Chen:** None. **M. Jackrel:** None. **C. Weihl:** None.

Poster

P313. Neuroinflammation: Sex Difference

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P313.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA 1R21DA039621–01
Hunkele Dreaded Disease Award
Samuel and Emma Winters Foundation
Charles Henry Leach II Fund
Commonwealth Universal Research Enhancement Award
Duquesne University Inaugural Provost's Interdisciplinary Research Consortia Grant
Beta Beta Beta Research Scholarship Fund

Title: Exploring the sex differences in neuropathic pain relief from a macrophage targeted COX-2 inhibiting nanotherapeutic

Authors: *B. S. DEAL[1,3], L. M. REYNOLDS[4,1,3], J. M. JANJIC[2,3], J. A. POLLOCK[1,3]; [1]Biol., [2]Pharm., Duquesne Univ., Pittsburgh, PA; [3]Chronic Pain Res. Consortium, Pittsburgh, PA; [4]Jefferson Univ., Philadelphia, PA

Abstract: Chronic pain effects almost a quarter of the population, but only 5% of those suffering from chronic pain have reported complete relief and satisfaction from their treatment. Because males have historically been the only sex used in pre-clinical research, the parallels in the development of a neuroinflammatory response and formation of pain in females is less understood. Currently, the macrophage is known to be a principal influencer in the development of neuroinflammatory pain. Macrophages give rise to neuroinflammatory pain via the activation of their cyclooxygenase 2 (COX2) enzyme which leads to the production of prostaglandin E_2 (PGE_2) influencing leukocyte infiltration. Our previous work with males has shown that rats experiencing mechanical allodynia caused by chronic constriction injury (CCI) of the sciatic nerve can achieve multi-day pain relief (equal to baseline) from a single injection of a novel nanotherapeutic containing celecoxib (0.24 mg/kg). The nanotherapeutic works by exploiting the natural neuroinflammatory role of macrophages. Once injected into the blood stream, monocytes phagocytose the nanotherapeutic and then accumulate at the site of injury where PGE_2 production is attenuated. This, in turn, results in a reduction of the number of infiltrating macrophages. Our current study shows that although males and females display quantitatively the same degree of mechanical allodynia post CCI, their responses significantly differ when treated with the same dose of celecoxib-loaded nanotherapeutic. Females exhibit an attenuated level of pain relief when compared to males. Females treated with the celecoxib nanotherapeutic

have a significant reduction in the number of infiltrating macrophages, but still have significantly more than the celecoxib treated males.

Disclosures: B.S. Deal: None. **L.M. Reynolds:** None. **J.M. Janjic:** None. **J.A. Pollock:** None.

Poster

P313. Neuroinflammation: Sex Difference

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P313.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1R03NS088395-01A1
NIH Grant 1R15NS093539-01
NIH Grant 1R21NS107960-01
The Michael J Fox Foundation for Parkinson's Research Rapid Response Innovation Award
Hillman Family Foundation Grant 109033
Center for Protein Conformational Diseases (University of Pittsburgh) Graduate Student Fellowship
NIH NeuroBioBank HHSN-271-201300029C Human Brain and Spinal Fluid Resource Center, VA West Los Angeles Healthcare Center, US Department of Veteran Affairs, and University of Miami Brain Endowment Bank

Title: Sex distinctions in the impact of microglia on preformed fibril-induced α-synucleinopathy

Authors: *T. N. BHATIA[1], R. N. CLARK[1], K. M. MINER[1], E. A. ECKHOFF[1], M. N. CHANDWANI[1], N. ABRAHAM[1], A. S. JAMENIS[1], X. HU[2], L. A. O'DONNELL[1], J. CHEN[2], K. C. LUK[3], R. K. LEAK[1];
[1]Div. of Pharmaceut. Sci., Duquesne Univ., Pittsburgh, PA; [2]Dept. of Neurol., Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept. of Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: It is well established that the substantia nigra displays microglial activation in Parkinson's disease subjects of both sexes. However, in the olfactory bulb (OB), Parkinson's-induced microgliosis is observed in male but not female subjects (Flores-Cuadrado *et al* 2021). In the postmortem OB of patients with dementia with Lewy bodies (DLB), we observed higher expression of microglial activation marker Iba1 in male (but not female) subjects compared to unaffected controls. However, the functional impact of these sex differences remains unknown. Here, we tested the impact of sex-stratified primary hippocampal microglia on α-synucleinopathy in sex-matched primary hippocampal neurons. Microglia from male rat pups (as determined by the *Sry* gene) reduced the numbers of α-synuclein^{+} inclusions, average sizes of

inclusions, and the total area occupied by inclusions in cocultured male neurons. Female microglia did not affect the numbers of inclusions or the total area occupied by the inclusions, and, unexpectedly, increased average inclusion sizes in cocultured female neurons. Preliminary data showed no robust sex differences in primary microglial uptake of $ATTO_{647}$-conjugated α-synuclein fibrils after 0, 6, 16, 24, 72, and 96 h. These observations suggest that male microglia mitigate inclusion formation better than female microglia and that these sex-dependent effects are not attributable to fibril uptake per se. Next, we hypothesized that *in vivo* microglial depletion for 2 weeks followed by repopulation for 6 weeks may affect deficits associated with α-synuclein fibril infusions. Eighteen month-old male and female mice were fed control chow or chow supplemented with 1200 mg/kg of PLX5622 – an inhibitor of the colony stimulating factor 1 receptor (CSF1R) known to deplete microglia. After 7d on this diet, we injected preformed fibrils into the rear of the mouse OB, as a preclinical model of limbic-centered α-synucleinopathy (Mason *et al.* 2016, 2019). We performed the open field test to assess hyperactive, exploratory, and anxiety-like behaviors 14d after microglial depletion (immediately prior to repopulation). Microglial depletion increased hyperactivity (*i.e.,* distance traveled and speed) in mice of both sexes. Microglia-depleted females showed an increase in the number of central square entries and the time spent within central squares. In contrast, male mice preferred the corner squares after microglial depletion. These data may reflect baseline sex differences in the impact of microglial depletion. Ongoing studies are testing the impact of microglial repopulation on pathology and neurological deficits in this animal model.

Disclosures: T.N. Bhatia: None. **R.N. Clark:** None. **K.M. Miner:** None. **E.A. Eckhoff:** None. **M.N. Chandwani:** None. **N. Abraham:** None. **A.S. Jamenis:** None. **X. Hu:** None. **L.A. O'Donnell:** None. **J. Chen:** None. **K.C. Luk:** None. **R.K. Leak:** None.

Poster

P313. Neuroinflammation: Sex Difference

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P313.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA Grant R01AG062716

Title: Impact of sex and age on cognitive deficits and inflammatory profile following a lipopolysaccharide induced immune challenge.

Authors: *J. DARLING, A. PRABHAKAR, S. GRANT, R. ANIRUDHAN, C. MANNERS, L. FONKEN;
Univ. of Texas at Austin, Austin, TX

Abstract: Aging is a major risk factor for cognitive decline. Neuroinflammation, which can contribute to cognitive decline, is modulated in aged individuals. Biological sex regulates the onset and severity of various neurodegenerative diseases that involve neuroinflammation including Parkinson's disease, Alzheimer's disease, and multiple sclerosis. Here we determined the impact of aging and sex on cognitive function and inflammatory profile in rats following a lipopolysaccharide (LPS) immune challenge. Young adult (3 month) and aged (24 month) male and female F344xBN rats received an intraperitoneal injection of 40μg of LPS or vehicle (saline) and then were trained and tested in a contextual fear conditioning paradigm. Briefly, 48h following LPS exposure, rats were placed into a novel chamber and received a 2 sec, 1.5mA shock through a floor grid. 48 hours later, rats were returned to the chamber and contextual fear memory (presented as time spent freezing) was measured. LPS exposure led to a reduction in freezing in aged male rats as compared to young LPS males and age matched saline controls, indicating an immune challenge induced sustained impairment in contextual memory. In contrast, no significant change in freezing behavior occurred in aged females relative to age matched saline controls nor young adults. Differences in cognitive function may relate to neuroinflammatory changes induced by microglia: microglia isolated from the hippocampus of aged male but not female rats exhibit exaggerated pro-inflammatory cytokine responses to ex vivo LPS challenge. Moreover, aged male but not female rats exhibit potentiated hippocampal IL-1β mRNA and protein 6, 24, and 48h following LPS exposure. These results suggest aged male rats are at higher risk for cognitive dysfunction following neuroimmune challenge versus females. This work highlights the importance of inclusion of sex as a variable when investigating age-related disorders. Ongoing investigation will further determine the mediating mechanisms underlying this age-related sex difference in behavior.

Disclosures: J. Darling: None. **A. Prabhakar:** None. **S. Grant:** None. **R. Anirudhan:** None. **C. Manners:** None. **L. Fonken:** None.

Poster

P313. Neuroinflammation: Sex Difference

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P313.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Connecticut Children's Medical Center Fellows Fund

Title: Sex differences in the protective mechanism of action of caffeine on microglia in a rodent model of preterm hypoxic ischemic injury

Authors: *R. M. MCLEOD[1], R. KOSKI[2], T. ROSENKRANTZ[3], R. H. FITCH[1];
[1]Univ. of Conneticut, Storrs, CT; [2]Univ. of Minnesota Med. Shcool, Minneapolis, MN;
[3]Univerity of Connecticut Hlth., Farmington, CT

Abstract: Currently 11% of infants are born prematurely, and a substantial portion experience hypoxic ischemic (HI) injury (i.e., transient or chronic reduction in blood and/or oxygen supply to the brain). Such events can lead to deficits in motor and cognitive ability. Though no neuroprotective treatments are approved for preterms, caffeine is routinely used to treat apnea of prematurity. This drug has also shown benefits to cognitive outcomes in premature infants, as supported by animal studies. Despite accumulating evidence of neuroprotective benefits, it is unclear *how* caffeine exerts protective effects in the preterm brain. Caffeine is a known adenosine antagonist, but it is unknown if adenosine acts directly on neurons (e.g., to prevent apoptosis), as an indirect anti-inflammatory, or on other targets like microglia. It is also unclear whether A1 and/or A2A receptors (expressed in neurons/microglia) modulate effects. Further, little is known about variables that may *modulate* caffeine action (e.g., sex, gestational age, or comorbid infection/inflammation). For example, evidence shows that female preemies have better outcomes than males, and also that hypothermia is more effective for neonatal females with HI, based on animal research (Wood et al., 2020). Thus sex might also modulate protection from caffeine. The current study investigated the role of microglia in caffeine-mediated neuroprotection in both neonatal male and female rats with HI, following a model of a 32-week gestational age HI injury (Rice-Vannucci method). Male and female rats with HI or sham surgery were further treated with caffeine or saline. All subjects were perfused 48 hours post injury. Brains were sectioned and stained for chromatin condensation, an indicator of cell death, using 4',6-diamidino-2-phenylindole (DAPI), as well as for microglia using ionized calcium binding adaptor molecule 1 antibody (IBA-1, which binds specifically to microglia). Results demonstrated increased chromatin condensation in both HI-saline and HI-caffeine rats relative to shams, as well as reduced cell death in caffeine-treated HI vs HI-saline subjects. However, females did ***not*** show reduced microglia activation (increased microglial soma size), which was seen in caffeine-treated HI males. This disparity points to differences in the neuroprotective mechanisms of caffeine, which may be of clinical significance. Work was supported by CCMC Fellows Fund.

Disclosures: R.M. McLeod: None. **R. Koski:** None. **T. Rosenkrantz:** None. **R.H. Fitch:** None.

Poster

P313. Neuroinflammation: Sex Difference

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P313.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5U01NS103882

Title: Sex-specific gene expression profiles of neonatal rabbit brain diminish with inflammation and dendrimer-N-acetylcysteine therapy in a cerebral palsy model

Authors: *A. FOWLER[1], N. SAH[1], A. CHIME[1], E. SMITH KHOURY[1], A. SHARMA[2], R. KANNAN[2], S. KANNAN[1];
[1]Anesthesiol. and Critical Care Med., [2]Ctr. for Nanomedicine, Dept. of Ophthalmology, Wilmer Eye Inst., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Sex plays an important role in the occurrence and severity of cerebral palsy (CP). According to epidemiologic data, incidence of CP in males is 30% higher compared to females. To understand the mechanisms behind conspicuous sex-specific susceptibility to CP, we studied the gene expression profile of male and female neonatal rabbit brain in a model of maternal-inflammation induced cerebral palsy. Furthermore, we compared the gender-specific therapeutic effects of dendrimer-N-acetylcysteine (DNAC), a potent antioxidant and anti-inflammatory drug, at the transcriptional level. Significant differences are observed in the transcriptome of healthy male and female brains, possibly originating from neuron and glia exposed to the perinatal surge of sex steroids. Endotoxin-exposed kits showed similar sexually dimorphic gene expression pattern, however, differences decreased between the male and female compared to healthy control. Surprisingly, DNAC administration changed the transcriptome profile of brain cells in both male and female CP kits such that sexual dimorphism is abolished to a large extent. Since DNAC uptake is primarily in glial cells in the brain, it's intriguing to speculate that glial cells and its oxidative state might shape sexual dimorphism. Overall, our study provides a basis for sexual bias in cerebral palsy susceptibility and therapeutic outcomes, that might necessitate gender-specific treatment paradigms.

Disclosures: A. Fowler: None. **N. Sah:** None. **A. Chime:** None. **E. Smith Khoury:** None. **A. Sharma:** None. **R. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics. **S. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.01

Topic: C.10. Brain Injury and Trauma

Support: VA - I01 RX001776-05

Title: Dysfunction of the descending pain-modulation system is involved in the augmented pain response after traumatic brain injury

Authors: *Q. CHEN, J. CLARK;
Dept. of Anesthesiology, Pain and Perioperative Med., Stanford Univ., Stanford, CA

Abstract: Individuals with histories of traumatic brain injury (TBI) commonly exhibit prolonged recovery after peripheral injuries and experience chronic pain. Recent laboratory and clinical data suggest dysregulated central pain-modulating circuits could be involved. We hypothesized that TBI exacerbates pain in response to a subsequent soft tissue injury by virtue dysfunctional descending pain-modulation. In a mouse model of mild TBI, we observed prolonged allodynia in the bilateral hindpaws of animals with TBI lasting up to 10 days. When hindpaw incisions were made in control and TBI mice after recovery of normal pain thresholds, mechanical thresholds in control mice returned to baseline after 7 days, while those animals with prior TBI experienced prolonged sensitization lasting up to 38 days. Furthermore, blockade of central but not peripheral mu opioid receptors (MORs) led to mechanical sensitization in both hindpaws in animals recovered from TBI. Control animals and those with incision alone did not experience changes in their pain thresholds after MOR blockade. Increased neuronal activation, marked by c-Fos staining, was observed in brain regions important for descending pain-modulation, such as rostral ventrolateral medulla, in animals recovered from TBI after MOR blockade. Collectively, these data suggest that dysfunctional descending pain-modulation is responsible for the slow resolution of pain after peripheral injury in the setting of TBI. The endogenous opioid system is important for the subsequent recovery of pain sensitivity after TBI but not after peripheral injury alone. Identifying ways to enhance endogenous pain control mechanisms may help those with pain after TBI.

Disclosures: Q. Chen: None. **J. Clark:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.02

Topic: C.10. Brain Injury and Trauma

Support: NIGMS/NIH COBRE II
NIG Grant NS119991
RCMI 8G12MD007600
Puerto Rico Center Clinical Translational Research Consortium
Brain and Behavior Research Foundation (NARSAD)

Title: Closed-head injury increased avoidance in rats

Authors: *O. MARTÍNEZ GUZMAN, M. CACERES-CHACON, M. RIVERA-LÓPEZ, H. HADDOCK-MARTINEZ, D. SIERRA-MERCADO;
Anat. and Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Closed-head injury increased avoidance in rats
Osmarie Martínez-Guzmán[1], Mauricio Cáceres-Chacón[1], Melissa Rivera-López[1], Héctor Haddock-Martínez[2], and Demetrio Sierra-Mercado[11]Department of Anatomy & Neurobiology, University of Puerto Rico (UPR) School of Medicine; [2]UPR Piedras
The most common form of brain injury, concussion, is frequently seen in contact sports and military combat. Converging lines of evidence suggest that concussion may impair fear-related behaviors. One type of fear-related behavior, avoidance, occurs when the need to escape from difficult situations such as an aversive stimulus (i.e. footshock) is presented in the presence of a reward (i.e. sucrose pellets). The effects of concussion on avoidance remains unknowns. Concussion can be modeled in rodents with a closed head injury (CHI). Here, a guide tube is placed above the head of anesthetized rats, and a weight is dropped through the tube. In the current study, we hypothesize that CHI will impair avoidance. One hour after CHI or Sham injury, locomotion behaviors were assessed in an open field to test for motion deficits, and CHI did not affect the distance travelled. In the platform-mediated avoidance, rats were conditioned in an operant chamber to auditory tones co-terminating with a mild footshock. An acrylic platform in the opposite corner of the sucrose-delivering bar allowed rats to avoid the shocks. Animals that underwent a CHI spent more time on the platform throughout the test session during the absence of the tone, even though they learn previously that the absence of tone was a safe period. These suggesting that brain injury results in excess avoidance ($p=0.0127$). The translational relevance of this work suggests that brain injury may contribute to mental health disorders, since excess avoidance is characteristic of patients with fear and anxiety disorders.

Disclosures: O. Martínez Guzman: None. **M. Caceres-Chacon:** None. **M. Rivera-López:** None. **H. Haddock-Martinez:** None. **D. Sierra-Mercado:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.03

Topic: C.10. Brain Injury and Trauma

Support: Detroit VA Medical Center, VA award RX-003198 (KEB)
Detroit VA Medical Center, VA award RX-003267 (ACC)
Wayne State University Undergraduate Research Opportunities Program UROP (NA, ACC)
Richard Barber Interdisciplinary Research Program (JIM and ACC)

Title: Effects of photobiomodulation light therapy (PBM) on traumatic brain injury-induced opioid seeking

Authors: *N. ALESAWY[1], S. LLOYD[3], J. MALEWICZ[2], K. E. BOSSE[3], A. C. CONTI[4]; [1]Neurosci., [2]Wayne State Univ., Detroit, MI; [4]Translational Neurosci. Program, [3]Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: While traumatic brain injury (TBI) has extensively been identified as a risk factor for development of substance use disorder related to nicotine, alcohol and cocaine, comparably less has been established for its relationship with opioids. However, understanding this association is imperative since over 70% of TBI patients suffer ongoing pain and are five times more likely to be prescribed opioids for pain management, which could lead to increased addiction liability in those with TBI, potentially through mechanisms involving oxidative stress and neuroinflammation, which are key pathological consequences of both TBI and opioid action. Photobiomodulation (PBM) is a potent, noninvasive light therapy useful in treating pain, inflammation and wound healing with known effects to mitigate oxidative and inflammatory signaling, making it safer therapeutic option. We hypothesized that TBI-induced opioid seeking behavior would be diminished by PBM through its anti-inflammatory and oxidative stress reduction mechanisms. Male, 10-12 week-old C57BI/6 mice were randomly assigned to receive a midline, closed-skull impact injury or sham surgery (n=6/condition). At 30 days post-TBI, voluntary consumption was measured in a two-bottle choice, 24-hour free access model with initial access (four daily sessions) to morphine (0.1 mg/ml in 0.2% sucrose) opposed to a quinine (0.02 mg/ml in 0.2% sucrose) solution, during which average morphine consumption was comparable across injury groups (sham: 16.4 ± 2.1 mg/kg; TBI: 15.4 ± 1.8 mg/kg). When quinine was substituted for the vehicle sucrose solution, morphine drinking over four daily sessions remained unchanged in TBI mice (14.5 ± 1.6 mg/kg), but markedly diminished in sham subjects (6.9 ± 1.8 mg/kg). A single session of PBM exposure (Quantum Devices, 670 nm LED; 50 mW/cm2; 90 sec) significantly attenuated the potentiated morphine intake in the TBI mice. Subsequent PBM exposure, repeated daily, reduced morphine consumption in TBI mice, while intake by sham controls was unaffected. Together, these data suggest that PBM is effective in reducing post-TBI induced opioid seeking behavior and may represent an effective treatment for opioid misuse after TBI in the clinical population. Supported by facilities at the Detroit VA Medical Center, VA awards RX-003198 (KEB) and RX-003267 (ACC), the Richard Barber Interdisciplinary Research Program (JIM, ACC) and the Wayne State University Undergraduate Research Opportunities Program (NA, ACC).

Disclosures: **N. Alesawy:** 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.; Wayne State University Undergraduate Research Opportunities Program UROP. **S. Lloyd:** None. **J. Malewicz:** 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.; Richard Barber Interdisciplinary Research Program. **K.E. Bosse:** 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.; Detroit VA Medical Center, VA award RX-003198 (KEB). **A.C. Conti:** 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.; Detroit VA Medical Center, VA awards RX-003267 (ACC).

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.04

Topic: C.10. Brain Injury and Trauma

Support: VA BLR&D 1IK2BX003258NIAAA
VA Seed (AGS)
The Department of Veterans Affairs Office of Research and Development
Medical Research Service (DGC)
VA Rehabilitation Research & Development Service (ERP)
University of Washington Friends of Alzheimer's Research (DGC, ERP)
UW Royalty Research Fund (DGC)
Northwest Network Geriatric Research, Education and Clinical Center (DGC)

Title: The Dynorphin/Kappa Opioid Receptor mediates adverse behavioral outcomes induced by repetitive blast mild traumatic brain injury

Authors: *S. LEE[1], B. BASKIN[3], M. YAGI[1], E. R. PESKIND[2,4], M. M. RASKIND[2,4], D. G. COOK[5,3,1,6], A. G. SCHINDLER[4,3,1];
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Abstract: Mild traumatic brain injury (mTBI), called the "signature injury" of Veterans of the wars in Iraq and Afghanistan, is highly comorbid with post-traumatic stress disorder (PTSD), depression, and chronic pain. Repetitive blast exposure is a common cause of these mTBIs with over 400,000 affected Veterans, yet effective treatment options are limited. Repetitive blast mTBI is often associated with aversion/dysphoria (a profound state of unease or dissatisfaction) mediated largely through activation of the endogenous dynorphin/kappa opioid receptor (KOR) system. Critically, trauma-induced KOR activation can exacerbate affective disorders and increase the risk for substance use disorder. However, little is known about the role KOR signaling plays in these behavioral symptoms of Veterans with a history of blast trauma nor in

animal models of blast. We and others have reported that mice exposed to repetitive blast exposure develop PTSD-like behaviors that include chronic aversion/dysphoria to previous blast-paired cues. Here, we hypothesized that the dynorphin/KOR system mediates blast-induced aversion/dysphoria. We evaluated this by pharmacologically antagonized KOR activation to block adverse blast-induced behavioral outcomes. We used a well-established pneumatic shock tube that models battlefield-relevant open-field blast forces generated by detonation of high explosives. Male mice were pretreated with vehicle (saline) or the selective KOR antagonist norbinaltorphimine (norBNI) prior to repetitive (3x) sham (anesthesia only) or blast exposure followed by behavioral testing at the one-month timepoint. KOR antagonism did not block blast-induced increase in loss of righting reflex or acute weight loss. Conversely, KOR antagonist pretreatment prevented blast-induced conditioned aversion, hyperarousal, social deficit, and anxiety/risk-taking/disinhibition but not sensorimotor deficits when tested one-month post sham/blast exposure. Our results show that KOR antagonist norBNI inhibited blast-induced affective, but not sensorimotor deficits in blast exposed mice. Together, these data suggest a new potential treatment strategy targeting the KOR system to prevent some chronic neuropsychological symptoms experienced by individuals with blast-related mTBI.

Disclosures: S. Lee: None. **B. Baskin:** None. **M. Yagi:** None. **E.R. Peskind:** None. **M.M. Raskind:** None. **D.G. Cook:** None. **A.G. Schindler:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.05

Topic: C.10. Brain Injury and Trauma

Support: Swedish Armed Forces

Title: Sex-dependent differences in hippocampal expression of Cytochrome P450 1B1 in penetrating traumatic brain injury.

Authors: *E. LIDIN[1], M. K. SKOLD[1,2], M. ANGÉRIA[1], J. DAVIDSSON[3], M. RISLING[1]; [1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Dept. of Neuroscience, Section of Neurosurg., Uppsala Univ., Uppsala, Sweden; [3]Div. of Vehicle Safety, Dept. of Mechanical and Maritime Sci., Gothenburg, Sweden

Abstract: Abstract Hippocampal dysfunction contributes to multiple traumatic brain injury sequala. Female rodents' outcome after injury is superior to male which has been ascribed the neuroprotective sex hormones 17β-estradiol and progesterone. Cytochrome P450 1B1 is an oxidative enzyme influencing the neuroinflammatory response by creating inflammatory mediators and metabolizing neuroprotective 17β-estradiol and progesterone. In this study, we

aimed to determine how penetrating traumatic brain injury influences hippocampal Cytochrome P450 1B1 mRNA expression and if sex-dependent differences exist. 64 adult Sprague Dawley rats equally divided by sex were subjected to penetrating traumatic brain injury or sham-surgery and assigned survival times of 1-, 3-, 5- or 7 days. mRNA in-situ hybridization was performed to quantify Cytochrome P450 1B1 mRNA in hippocampal subregions and immunohistochemical analysis to verify protein colocalization. Cytochrome P450 1B1 mRNA expression was greatest in CA2 irrespective of sex. 3-, 5- and 7 days post-injury, expression in CA2 was reduced in male rats subjected to penetrating traumatic brain injury compared to those receiving sham-surgery. Females subjected to penetrating traumatic brain injury instead exhibited increased expression in all CA subregions 3 days post-injury, the only time point expression in CA2 was greater in females than in males. Immunohistochemical analysis confirmed Cytochrome P450 1B1 protein in all hippocampal subregions. Following penetrating traumatic brain injury, Cytochrome P450 1B1 mRNA expression exhibits sex-dependent differences. Females displayed a more urgent but brief regulatory response than males. This indicates there may be sex-dependent differences in Cytochrome P450 1B1 activity, possibly influencing inflammation and neuroprotection in penetrating traumatic brain injury.

Disclosures: E. Lidin: None. **M.K. Skold:** None. **M. Angéria:** None. **J. Davidsson:** None. **M. Risling:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.06

Topic: C.10. Brain Injury and Trauma

Support: NHMRC 1173565

Title: Gut Microbiome Depletion in Adolescence Modifies Behaviour, Bone Development, and Neuropathology in a Rodent Model of Repetitive Mild Traumatic Brain Injury

Authors: M. SGRO[1], Z. KODILA-JONES[2], K. WONG[1], C. LI[1], R. D. BRADY[1], G. R. YAMAKAWA[1], ***R. MYCHASIUK**[1];
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Abstract: It is well recognized that the gut microbiome plays an integral role in optimal brain development and function. Recent literature demonstrates that mild traumatic brain injury (mTBI) alters the gut microbiome and the brain-gut axis, leading to persistent neuroinflammation. However, there has been no investigation into the effects that the microbiome may have on adolescent mTBI pathophysiology. Given that studies have

demonstrated that chronic antibiotic administration depletes the gut microbiome, antibiotic administration is a non-invasive means by which the microbiome can be manipulated within the context of mTBI. We therefore investigated the effects of microbiome depletion on behavioural recovery, bone development, and neuropathology following repetitive mTBI in adolescence. Specifically, adolescent rats (39F:40M) were administered an antibiotic cocktail or placebo in their drinking water for 14 days. The rats were then randomly assigned to receive 3 mild TBIs (RmTBI) or sham injuries followed by a behavioral test battery to examine functional recovery. The battery included rotarod for motor function, the elevated plus maze (EPM) for anxiety-like behaviour, the hot/cold plate for pain sensitivity, and play plus temperament scoring for social behaviour. We used micro-computed tomography (μCT) to examine bone volume, bone density, and femur length. Finally, immunohistochemistry was used to investigate changes in perineuronal net integrity, as well as microglia activation within the prefrontal cortex. Analyses of fecal samples demonstrated that antibiotic administration effectively depleted the gut microbiome. Three-way ANOVAs with sex (Female/Male), treatment (Antibiotic/Standard), and Injury (RmTBI/Sham) as factors were run. We found that microbiome depletion and RmTBI differentially modified motor, anxiety-like, pain, and social behaviours, in a sex-dependent manner. In addition, we found sexually dimorphic changes in adolescent bone development. For example, female femur length was reduced in response to both RmTBI and microbiome depletion (but not when combined), while male femur length was only restricted by microbiome depletion. Finally, using a triple label for Wisteria floribunda agglutin, parvalbumin neurons, and IBA1, we demonstrate alterations to perineuronal net integrity surrounding parvalbumin interneurons and microglia activation that are dependent upon injury, microbiome depletion, and sex. These results suggest that the microbiome modulates recovery from RmTBI and may be an efficacious therapeutic target for neurotrauma if the treatment is differentially formulated for males and females

Disclosures: M. Sgro: None. **Z. Kodila-Jones:** None. **K. Wong:** None. **C. Li:** None. **R.D. Brady:** None. **G.R. Yamakawa:** None. **R. Mychasiuk:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.07

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 NS 111378 and NS 111378-S
NIH R01 NS 117148
NIH R01 NS 050465
NIH R01 NS 116838

Title: Traumatic brain injury alters gut-derived peripheral serotonin signaling in mice

Authors: *N. M. MERCADO, G. ZHANG, Z. YING, F. GOMEZ-PINILLA;
Dept of Integrative Biol. & Physiol., UCLA, Los Angeles, CA

Abstract: Most efforts to understand the pathology of traumatic brain injury (TBI) have been centered on the brain, ignoring the role played by systemic physiology in TBI and limiting understanding of the dimensionality of pathogenesis and treatment efficacy. Gut-derived serotonin (5-HT), which interacts with gut microbiota and various tissues throughout the body under the regulatory action of the enteric nervous system (ENS), is emerging as a major regulator of whole body homeostasis and energy balance. We are devoting major efforts to understand the action of TBI on systemic function via regulation of peripheral 5-HT signaling. Male C57BL/6J mice were subjected to a mild fluid percussion injury (FPI; 1.5-1.7 atm) and compared to sham animals. Mice were sacrificed 1, 3, or 7 days following FPI. Reverse-transcription qPCR was used to examine gene expression changes following TBI in intestine, liver, and adipose tissue. Systemic 5-HT levels were measured in blood by ELISA and visualized in colon using immunofluorescence. In the ENS, neuronal markers *Chat* and *Nos* were transiently downregulated in the duodenum and colon following TBI. In the colon, alterations in genes related to synthesis and degradation of 5-HT were observed in TBI mice vs sham, favoring an overall 5-HT downregulation. Colonic 5-HT expression corroborated these findings, showing a significant decrease in 5-HT fluorescence intensity at 7 days post-TBI. Blood 5-HT levels were also significantly decreased in TBI mice compared to sham at this time point. Furthermore, we detected alterations in 5-HT-associated gene expression in downstream tissues after TBI: namely, upregulation of 5-HT receptor *Htr2a* in liver and adipose, and upregulation of lipogenesis marker *Srebp1* in liver. Our findings suggest that TBI alters peripheral 5-HT homeostasis by lowering systemic 5-HT levels, a change which has the potential to impact whole body energy balance and gastrointestinal function. In all, these data highlight the importance of building an integrative view of the role of systemic physiology in TBI pathogenesis, and we posit that a thorough understanding of the dimensionality of peripheral TBI pathology is necessary for the design of effective treatments.

Disclosures: N.M. Mercado: None. **G. Zhang:** None. **Z. Ying:** None. **F. Gomez-Pinilla:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.08

Topic: C.10. Brain Injury and Trauma

Support: ARTN; 2T32 AA14127-12
K22 NS092767

NCI P30CA118100
ACSIRG# 131567
UNM Grant Pilot Project #1541

Title: Loss of bHLH Transcription Factors Decreases Glioblastoma Progression and Increases Survival

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[2]Neurosciences, [3]Undergraduate Pipeline Network, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM

Abstract: Loss of bHLH Transcription Factors Decreases Glioblastoma Progression and Increases SurvivalGlioblastoma (GBM) makes up about 50% of primary brain tumors and survival has not increased over the last 30 years, with less than 5% of patients surviving past 5 years of diagnosis. The lethality of GBM is likely due to high degree of inter- and intratumoral heterogeneity and the presence of key neurodevelopmental transcription factors such as ASCL1 and OLIG2, which have been shown to be present in the majority of GBMs. Functionally, these basic-helix-loop-helix (bHLH) transcription factors form heterodimers with E-proteins (E47/TCF4) and bind to DNA to regulate gene transcription. Previously, we showed in patient-derived GBM xenograft (PDX-GBM) that ASCL1 binds to promoter and enhancer regions of cell cycle, mitotic, and chromatin organization genes, as well as neurodevelopmental transcription factors including that of *OLIG2*. Similarly, in this study we showed that OLIG2 is able to bind to the majority of ASCL1 target genes, including the promoter and/or enhancer regions of both *ASCL1* and *OLIG1/2* loci, illustrating a potential redundant and/or feed-forward function between these two bHLHs in GBM. It has been proposed that the presence of ASCL1 and OLIG2 contribute to the neural stem cell-like properties of these cancer cells, which may be responsible for the increased treatment resistivity and high recurrence rate of GBMs in patients. However, to date the combinatorial roles of these two bHLHs in GBM are as of yet untested in vivo in the brain. Remarkably, we found that the loss of ASCL1 or OLIG2 alone slows tumor progression but is not enough to stop tumor growth entirely in a glioma mouse model in which tumors are induced from glial progenitors in the brain of immune competent mice. In contrast, the combined loss of both ASCL1 and OLIG2 completely prevents tumor growth and results in 100% survival of mice up to 6 months. These research findings illustrate the need for a better understanding of the combinatorial function of ASCL1 and OLIG2 as well as their shared downstream targets as they could lead to the development of novel therapeutics for patients suffering from GBM.

Disclosures: B. Myers: None. **M. Keith:** None. **R. Anderson:** None. **T. Vue:** None.

Poster

P345. Animal Models of Brain Injury: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P345.09

Topic: C.10. Brain Injury and Trauma

Support: R01 NS091218

Title: Autophagy Dysregulation Exaccerbates NeuroInflammation Following Experimntal TBI in Mice

Authors: *N. HEGDEKAR[1], C. SARKAR[1], R. M. RITZEL[1], S. BUSTOS[1], M. HANSCOM[2], D. J. LOANE[2], A. FADEN[1], M. M. LIPINSKI[1];
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Abstract: The autophagy-lysosomal pathway serves an important role in cellular homeostasis and protection against neurodegeneration. Recently autophagy has been also implicated in regulation of immune and inflammatory responses. Specifically, high levels of autophagy flux - the progress of substrates through autophagic compartments leading to their delivery and degradation in the lysosomes - are generally associated with anti-inflammatory, and inhibition of flux with pro-inflammatory phenotypes. To determine if autophagy may be involved in modulation of neuroinflammation after traumatic brain injury (TBI), we assessed the levels of autophagy in resident microglia and infiltrating macrophages following moderate controlled cortical impact (CCI) in mice. Our data demonstrated accumulation of autophagosomes and inhibition of autophagy flux specifically in the activated microglia and infiltrating macrophages starting by day 1 and continuing through day 28 post-CCI. Our immunofluoresnce studies in transgenic *Cx3Cr1-GFP* microglial and *Ccr2-RFP* monocyte reporter mice confirmed inhibition of autophagy flux and demonstrated that while both activated resident microglia and infiltrating macrophages are affected, inhibition of autophagy is much more prominent in macrophages. Our FACS analysis demonstrated that immune cells with inhibited autophagy flux after CCI expressed increased levels of pro-inflammatory markers, including IL-1β and TNF-α, as compared to corresponding immune cells with normal levels of autophagy. Furthermore, inhibition of autophagy flux correlated with impaired phagocytic function, indicating microglial/macrophage dysfunction. These trends persisted through day 28- post CCI, suggesting that autophagy flux impairment following TBI has long-term consequences. *In vitro* experiments in IMG microglial and RAW macrophage cell lines demonstrated that inhibition of autophagy can potentiate pro-inflammatory activation induced by lipopolysaccharide (LPS) treatment. Our *in vivo* studies further support the importance of autophagy in inflammation regulation. Mice with microglia/macrophage-specific knockout of the autophagy gene *Beclin1* (*Beclin1-flox/LysM-Cre*) showed increased expression of pro-inflammatory gene and protein markers, and in particular, marked exacerbation of innate immune responses, including activation of the NLRP3 inflammasome and the type I IFN pathways and performed worse than *LysM-Cre* controls on behavioral tasks after CCI. Overall, these findings indicate that impairment of autophagy in microglia/macrophages exacerbates neuroinflammation after TBI and contributes to functional deficits.

Disclosures: N. Hegdekar: None. **C. Sarkar:** None. **R.M. Ritzel:** None. **S. Bustos:** None. **M. Hanscom:** None. **D.J. Loane:** None. **A. Faden:** None. **M.M. Lipinski:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 NS103350-01A1

Title: Protein kinase C δ is a molecular mechanism for headache in a mouse model of migraine

Authors: *Y. HE[1], R. O. MESSING[2], Z. J. WANG[3];
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Abstract: Migraine is one of the most common neurological disorders characterized by recurrent attacks of typically throbbing and unilateral headaches, affecting up to 20 % of the population worldwide. Despite the high prevalence and severity of this primary headache disorder, it remains to be a challenge to fully understand and treat migraine headaches. By characterizing and validating a mouse migraine model, this study aimed to investigate the functional contribution of PKC isoforms in migraine. In this study, we identified the presence of migraine-like ongoing pain in mice after chronic intermittent treatment with nitroglycerin (NTG). The peptide antagonist of calcitonin gene related peptide (CGRP) α-CGRP (8-37), but not topiramate nor sumatriptan, effectively blocked ongoing pain and elicited pain relief-induced CPP in NTG-treated mice. Prominent activation of PKCδ was observed in chronic NTG-treated mice. Functional inhibition of PKCδ significantly attenuated ongoing spontaneous pain in chronic NTG-treated mice. Furthermore, we recapitulated the NTG-triggered migraine behavior in wild-type, but not in PKCδ-null mice. In response to repeated administration of NTG, ongoing spontaneous pain was not developed in mice lacking the specific PKC isoform. This study identified the presence of ongoing pain in mice treated with nitroglycerin, a known human migraine trigger that closely resembles the common manifestation of spontaneous migraine attacks in humans. These findings demonstrated a critical regulatory role of PKCδ in migraine pathophysiology, which may offer new pharmacological targets for anti-migraine treatment.

Disclosures: Y. He: None. **R.O. Messing:** None. **Z.J. Wang:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant P01AT009965
NARSAD Young Investigator Grant 26236

Title: Effects of respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) on the modulation of cardiovagal response to trigeminal somatosensory stimulation in migraine patients

Authors: M. HYMAN[1], R. BARBIERI[4,2], R. R. EDWARDS[5,6], N. HADJIKHANI[1,6], R. GOLLUB[3,6,1], V. NAPADOW[1,6,5], *R. G. GARCIA[3,6];
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Abstract: Background & Aims: Hypersensitization of the brainstem trigeminal sensory complex may underlie impaired sensory processing and decreased cardiovagal regulation in patients with migraine. Recently, our group has developed a novel respiratory-gated transcutaneous auricular vagus nerve stimulation technique (RAVANS taVNS) aimed at the regulation of key brainstem nuclei involved in trigeminal somatosensory processing and autonomic activity. As taVNS has shown promise for episodic migraine in clinical trials, we evaluated the impact of RAVANS on modulation of cardiovagal response to trigeminal afference in episodic migraine patients. **Methods**: We enrolled 16 interictal episodic migraine patients (35.8±13.4 yrs, 15 F) and 16 age- and sex-matched healthy controls (36.0±13.7 yrs). Subjects received exhalatory-gated RAVANS and sham stimulation in a randomized, crossover design study. Aversive air-puff stimuli (14-sec ON and 20-sec OFF, 11 reps) were administered to the right supraorbital forehead (ophthalmic (V_1) trigeminal division) before and after a 6-min period of RAVANS or sham stimulation at the left cymba concha. Cardiac pulse was recorded, and a point process model calculated instantaneous estimates of the high-frequency power component of heart rate variability (HF-HRV), a measure of cardiovagal response. Peri-stimulus plots were obtained by averaging HF-HRV epochs temporally aligned with respect to stimulus onset (Fig. 1). The average HF-HRV power over the 5 seconds of pre-stimulus time was used as a baseline. **Results**: Migraineurs showed increased HF-HRV during forehead air-puff stimulation post-RAVANS compared to pre-RAVANS (p=0.04, Fig. 1). No significant changes in HF-HRV were observed when comparing post vs pre-RAVANS in healthy subjects, nor post vs pre-sham in either group. **Conclusion**: RAVANS effectively increased cardiovagal response to trigeminal sensory stimulation in migraineurs, which may constitute both an adaptive mechanism for the processing of trigeminal afference and a potential biomarker for clinical efficacy in this population.

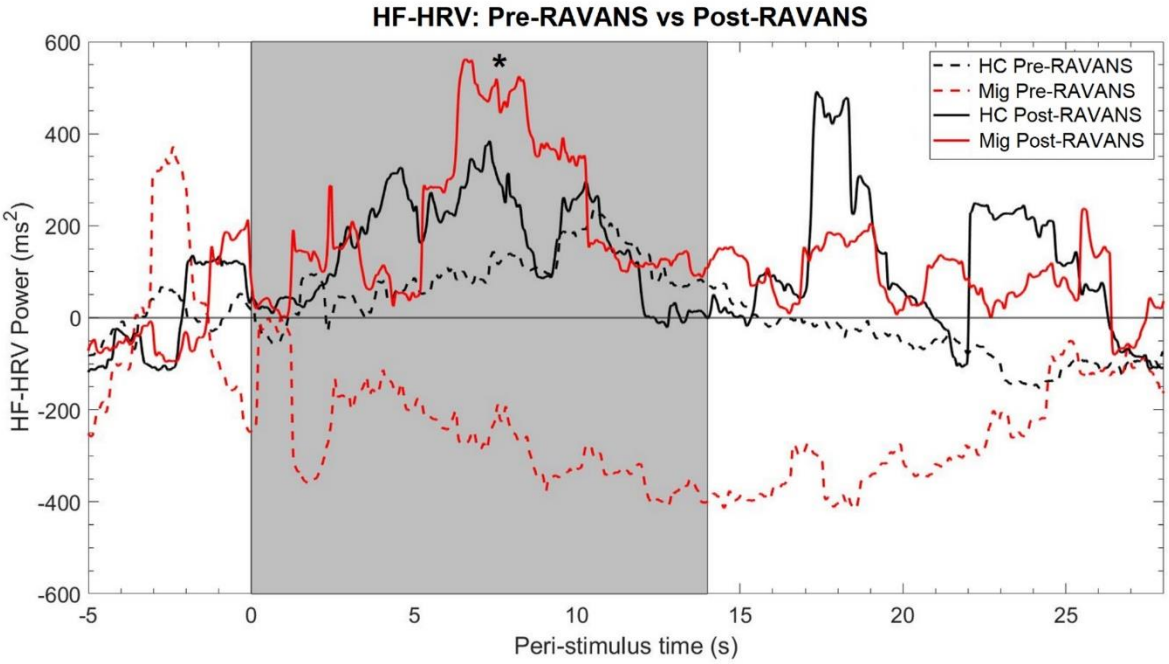


Figure 1. Peri-stimulus plot for HF-HRV changes during air-puff stimulation pre- and post-RAVANS in healthy controls (HC) and migraineurs (Mig)

Disclosures: M. Hyman: None. **R. Barbieri:** None. **R.R. Edwards:** None. **N. Hadjikhani:** None. **R. Gollub:** None. **V. Napadow:** F. Consulting Fees (e.g., advisory boards); Cala Health Inc.. **R.G. Garcia:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.03

Topic: D.03. Somatosensation – Pain

Support: NS099292

Title: Prophylactic Administration of Hsp90 Inhibitor 17-AAG Limits Onset of Trigeminal Facial Allodynia and Improves Blood Brain Barrier Integrity in KCl Model of Episodic Headache

Authors: *S. PALOMINO[1], A. LEVINE[1], J. WAHL[1], E. LIKTOR-BUSA[1], J. STREICHER[1], T. M. LARGENT-MILNES[2];
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Abstract: Blood-brain barrier (BBB) homeostasis is integral to the health and maintenance of the central nervous system. Dysregulation of the blood brain barrier has been associated with certain neuroinflammatory disease states such as stroke, Alzheimer's, and epilepsy. Migraine is the 3rd most prevalent disease in the world and the 6th ranked disease associated disability. Despite this fact there is limited data on blood brain barrier function and integrity during migraine states. Previously published data has shown that cortical spreading depression (CSD), a phenomenon related to migraine with aura, causes onset of trigeminal facial allodynia in rodents and compromises the physical integrity of the blood brain barrier. The neurogenic theory of migraine suggests both vascular and inflammatory components contribute to the pathology. Hsp90 inhibitors have been shown to have anti-inflammatory effects as well as neuroprotective action at the blood brain barrier in disease states such as stroke. In this study we observed the effects of Hsp90 inhibitor 17-AAG in a rodent model of KCl induced cortical spreading depression to assess pain outcome and barrier integrity and function. To investigate if Hsp90 inhibitors impacted the outcome of facial allodynia caused by cortical spreading depression we used a well characterized model of migraine with aura induced by cortical injection of KCl (1M, 0.5uL). Using immortalized mouse brain endothelial cells, Hsp90 inhibition with 17-AAG mitigated reductions in trans-endothelial electrical resistance induced by KCl. 17-AAG also decreased paracellular uptake of radiolabeled sucrose under the same conditions. Moreover, 17-AAG preincubation led to tight junction reorganization of claudin-5 in ICC images of Bend3 cells. In situ brain perfusions evaluated the effects of Hsp90 inhibition on the blood brain barrier 90 minutes post CSD. Results showed increased sensitization to pain with increasing doses of 17-AAG, however, at a moderate dose 17-AAG decreased the number of animals affected by cortical spreading depression. 17-AAG improved blood brain barrier integrity invitro and invivo. Studies are ongoing at this time to determine how 17-AAG application will affect sumatriptan activity.

Disclosures: S. Palomino: None. **A. Levine:** None. **J. Wahl:** None. **E. Liktor-Busa:** None. **J. Streicher:** None. **T.M. Largent-Milnes:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01NS075599-07

Title: The role of dural immune cells and blood vessels in a preclinical mouse model of migraine.

Authors: *L. BALCZIAK[1], M. CONRAD[1], W. CHO[3], A. KUBURAS[1], M. W. CHAPLEAU[2], A.-S. WATTIEZ[1], A. F. RUSSO[1];
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Abstract: Migraines are the leading cause of disability in the US and affect around 1 billion people worldwide. Symptoms include pain, photophobia, nausea and vomiting. The neuropeptide calcitonin gene-related peptide (CGRP) is a key mediator of migraine and drugs that block CGRP action are now being used to prevent migraine. However, the mechanism by which CGRP causes migraine remains unknown. It is thought that the site of migraine pain originates in the dura mater, specifically the trigeminal afferent endings around blood vessels and immune cells. CGRP released in this region from trigeminal neurons may cause dysregulation in the meningeal environment and cause sensitization of nearby afferents. Since CGRP causes vasodilation, immune cell activation and nociception, it is critical to parse out which effect may drive migraine. We previously showed that CGRP's systemic vascular effect isn't necessary for the migraine-like symptoms of light aversion. The current focus is on local dural vasculature and resident immune cells. We use a preclinical mouse model of migraine measuring migraine-like symptoms of light aversion (photophobia) and periorbital allodynia (pain) induced by CGRP. We found that $Kit^{+/-}$ mice with reduced mast cells may have a lower magnitude of CGRP-induced light aversion compared to controls. These mice and those that overexpress the CGRP receptor subunit hRAMP1 in monocyte lineage are currently being tested with periorbital von Frey. In vivo awake two-photon microscopy macrophage morphology is being investigated in the meninges along with meningeal blood vessel diameter. Additional work is being done to determine whether IP CGRP changes fluctuating serum cytokine levels during the normal behavioral testing window. This research should give insight into the potential interaction with the mouse dural immune system and meningeal vessels in a preclinical model of migraine to help flesh out understanding of CGRP's peripheral effects.

Disclosures: L. Balcziak: None. **M. Conrad:** None. **W. Cho:** None. **A. Kuburas:** None. **M.W. Chapleau:** None. **A. Wattiez:** None. **A.F. Russo:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.05

Topic: D.03. Somatosensation – Pain

Title: The Local Field Potential Change in Migraine Headache

Authors: *Z. WANG, Q. LIN, Y. B. PENG;
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Abstract: Migraine is a recurrent primary headache disorder and has been ranked as the second leading cause of disability and sixth prevalence disease with up to 1 billion patients worldwide. However, the pathophysiology of migraine headache remains not fully understood. The ***purpose*** of this study is to determine differential local field potential (LFP) changes, a neurophysiological tool to investigate the combined neuronal activity, from multiple selected brain regions related to migraine-relevant pain networks, by a well-established migraine model, the intraperitoneal (i.p.) injection of nitroglycerin (NTG). The ***hypothesis*** is that these brain areas, which are involved in different orders of neurons in trigeminovascular system and pain processing, will exhibit different response patterns to migraine attacks. In this study, LFPs from the anterior cingulate cortex (ACC, related to emotional processing), posterior nucleus of the thalamus (Po), trigeminal ganglion (TG), and primary visual cortex (V1M) (involved in trigeminovascular system) were simultaneously recorded, during the light aversion behavior in freely-moving animals. Four electrodes were implanted into these brain areas in Sprague-Dawley rats (n=11). After one-week recovery, LFPs were firstly recorded for 30 minutes as the baseline, then NTG was injected (i.p. 10 mg/kg), and continued recording for four hours. Light aversion behavior test, which was performed for 30 minutes, the percentage of time in dark box was then calculated, was measured one day before the recording as the baseline, and immediately after NTG injection. The control group rats (n=10) received i.p injection of vehicle solution. The raw data of LFP was processed by power spectrum analysis in MATLAB. The powers of delta (0.1-3 Hz), theta (3-7 Hz), alpha (7-12 Hz), beta (12-30 Hz), and gamma (30-100 Hz) bands were normalized by the average of the baseline. A mixed analysis of variance (ANOVA) was implemented to test the difference of LFP and behavior changes between NTG and control groups. The results indicated that: Overall, the relative change of LFP powers for delta, theta, alpha, beta, and gamma bands were increased significantly ($p < .05$) after NTG injection (n=11) in the ACC, Po, TG, and V1M simultaneously in contrast to the control group (n=10). The light aversion test showed a significant elevation of time spent in the dark box in the NTG group ($p < .05$). In conclusion, NTG-induced migraine has significant LFP intensity in various related brain regions, indicating potential parameters for predicting migraine.

Disclosures: Z. Wang: None. **Q. Lin:** None. **Y.B. Peng:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.06

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS103350-02S1
Department of Defense Investigator-Initiated Research Award W81XWH1810627

Title: Enhanced CGRP and PACAP signaling in trigeminal ganglion neurons in mouse models of chronic migraine and post-traumatic headache

Authors: *Z. GUO, K. CZERPANIAK, J. ZHANG, Y.-Q. CAO;
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Abstract: Numerous studies indicate that calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating polypeptide (PACAP) signaling pathways contribute to migraine pathophysiology. Both CGRP and its receptors are abundantly expressed in primary afferent neurons in trigeminal ganglia (TG). Under normal condition, endogenous CGRP activates/sensitizes nociceptors through paracrine signaling. However, whether repeated headache episodes alter the relative contributions of autocrine versus paracrine CGRP signaling, or the overlap between CGRP and PACAP signaling in TG neurons have not been investigated. In this study, we used EGFP signal in heterozygous CGRP$\alpha^{EGFPf/+}$ mice to define TG neurons that express endogenous CGRP. TG neurons that exhibit functional CGRP and/or PACAP receptors were identified by CGRP- and/or PACAP-evoked intracellular Ca^{2+} increase. This allowed us to reveal the relationship between CGRP-responsive (CGRP-R), PACAP-responsive (PACAP-R) and CGRP-expressing neurons in mouse TG under normal and disease states. Under normal condition, most CGRP-R TG neurons did not express CGRP, indicating that paracrine CGRP signaling predominates. After repeated nitroglycerin (NTG, a reliable migraine trigger in migraineurs) administration, there was a significant increase in CGRP-R neurons in mouse TG but not DRG, supporting its relevance to chronic migraine. The percentage of CGRP-R neurons were increased in both $EGFP^+$ and $EGFP^-$ cells, suggesting that both autocrine and paracrine CGRP signaling are enhanced in TG neurons during chronic migraine. In $EGFP^+$ TG neurons that express endogenous CGRP, repeated NTG led to several fold increase in the number of neurons that respond to both CGRP and PACAP (CGRP-R&PACAP-R), suggesting that the release of endogenous CGRP can be enhanced by both CGRP and PACAP signaling pathways under chronic migraine condition. The numbers of CGRP-R, PACAP-R as well as CGRP-R&PACAP-R neurons were also increased by mild traumatic brain injury (mTBI). Low-dose interleukin-2 treatment, which the behavioral sensitizations related to chronic migraine and post-traumatic headache (PTH), also blocked the increase in both CGRP-R and PACAP-R TG neurons in mouse models. Taken together, our findings suggest that enhanced CGRP and PACAP signaling in TG neurons may play important role in the pathophysiology of chronic migraine and post-traumatic headache. Inhibition of both CGRP and PACAP signaling in TG neurons may be more effective in treating chronic migraine and post-traumatic headache than targeting individual signaling pathways.

Disclosures: Z. Guo: None. **K. Czerpaniak:** None. **J. Zhang:** None. **Y. Cao:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.07

Topic: D.03. Somatosensation – Pain

Support: R01NS113243
R21NS121946

Title: Transient receptor channel 4 (TRPC4) small-molecule inhibition alleviates migraine-like behavior in mice

Authors: *C. F. COHEN, S. LEE, T. BERTA;
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Abstract: Migraine is a common neurological disorder that is estimated to affect 15% of the world population, making it the third most prevalent disease worldwide. Yet migraine underlying mechanisms are poorly understood, and few treatment options exist for this disorder. Transient receptor potential channels are important mediators of itch and pain and are emerging therapeutic targets. We have recently demonstrated the role of the transient receptor potential channel 4 (TRPC4) in itch and how blockade of TRPC4 reduced psoriatic-itch and skin inflammation, potentially through inhibition of calcitonin gene-related peptide (CGRP) release from peripheral sensory neurons. Because release of CGRP from peripheral sensory neurons is a major mechanism of migraine, we asked whether TRPC4 is involved and may therefore represent a therapeutic target in migraine. First, we assessed whether TRPC1-7 are expressed in trigeminal peripheral sensory neurons innervating the head. We found a significantly higher expression of TRPC4 transcripts in trigeminal ganglia (TG) compared to other members of the TRPC family. Furthermore, we showed by immunohistochemistry that the majority of TRPC4 positive neurons in TG also expressed CGRP. Second, we used a well-characterized mouse model of migraine consisting of repeated injections of nitroglycerin (NTG), 10 mg/kg, to assess the therapeutic effect of the small-molecule inhibitor ML-204, a specific TRPC4 antagonist. Notably, mice injected with NTG developed mechanical hypersensitivity mimicking symptoms observed in migraineurs. Assessments of mechanical sensitivity by von Frey filaments showed that intraperitoneal injections of ML204 (40 μg per animal) significantly alleviated NTG-evoked mechanical hypersensitivity compared to vehicle control injections in both male and female mice ($n=5$-6/group; $p < 0.05$). Third, we explored the potential mechanism underlying this alleviation and found a significant decrease of CGRP protein levels in blood of both male and female mice treated with ML-204 ($n=5$-6/group; $p < 0.05$). Using sensory neuron cultures, we confirmed that activation of TRPC4 by Englerin A (a specific TRPC4 agonist) evoked rapid CGRP release, which was significantly reduced by ML-204 ($n=4$/group; $p < 0.05$). Collectively, our findings identify TRPC4 in DRG and TG neurons as a mediator of CGRP release and NTG-evoked migraine. Since a TRPC4 antagonist is already in clinical trials, we expect that this study will rapidly lead to novel and effective clinical treatments for millions of patients suffering from migraine.

Disclosures: C.F. Cohen: None. **S. Lee:** None. **T. Berta:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS104100

Title: The role of glucocorticoids in repetitive stress-induced migraine-like behaviors in mice

Authors: *Y.-Y. HU[1], R. SOUZA[2], C. K. MCINTYRE[3], G. O. DUSSOR[4];
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Abstract: Stress is one of the most common precipitating factors in migraine and it is identified as a trigger in nearly 70% of migraine patients. The nervous system responds to stressors by activating neuroendocrine mechanisms, like inducing glucocorticoid release to change behaviors. Although it has adaptive value, migraine headache may be an adverse consequence of this glucocorticoid response. We have shown previously that repeated restraint stress in mice (3 days, 2 hours each day) evokes migraine-like behavioral responses as well as priming to the nitric oxide donor sodium nitroprusside (SNP). In the present study, we used CD-1 male and female mice to investigate the effect of glucocorticoids on these restraint stress-induced migraine-like behaviors. To examine the role of glucocorticoids, we gave subcutaneous injections of metyrapone, an inhibitor of glucocorticoid synthesis, 30 minutes prior to each restraint stress exposure. Metyrapone attenuated both the initial restraint stress-induced migraine-like behaviors and priming to SNP in both male and female mice. These results indicate that migraine-like behaviors in this model are dependent on normal synthesis of corticosterone (CORT). To further investigate the role of glucocorticoids in this model, and to test whether CORT injections alone can mimic restraint stress-induced migraine-like behaviors, we administered subcutaneous injections of either 10mg/kg, 20mg/kg doses of exogenous CORT, or vehicle for 3 consecutive days in the absence of restraint stress. Daily CORT injections led to migraine-like behaviors although the pattern of response was different from restraint stress. Restraint stress induced facial hypersensitivity 24 hours after the last day of restraint, but CORT alone did not induce facial hypersensitivity until 72 hours after the last injection. Moreover, CORT injections did not cause any pain-like facial expressions. During the SNP-induced priming phase, CORT injection group and stress groups both showed facial hypersensitivity. Neither CORT injection group nor the restraint stress group showed any pain-like facial expression during the priming phase. These data indicate that corticosterone plays an important, but not exclusive role in the behavioral responses to repeated stress. Together, these results reveal that glucocorticoids are critical for the development of migraine-like behavioral responses following repeated stress in mice. Better understanding of where, when, and how glucocorticoids influence the nervous system in the

context of stress may provide important information for how stress contributes to migraine and may lead to new therapeutic approaches.

Disclosures: Y. Hu: None. **R. Souza:** None. **C.K. McIntyre:** None. **G.O. Dussor:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.09

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS103350-02S1

Title: Low-dose interleukin-2 reverses chronic headache-related behavioral and cellular sensitization

Authors: *J. ZHANG[1], K. CZERPANIAK[1], Z. GUO[1], X. LIU[1], J. UNSINGER[2], R. HOTCHKISS[2], Z. QIN[3], Y. CAO[1];
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Abstract: Chronic headache disorders, including chronic migraine and post-traumatic headache (PTH), affect a large population and cause debilitating pain, with limited treatment options. Although a large body of work indicates that many pro-inflammatory immune cells contribute to headache pathophysiology, the involvement of the immuno-suppressive regulatory T (Treg) cells in the chronification of headache disorders remains unknown. Here, we used two mouse models to examine the potential therapeutical effect of Tregs in chronic headache disorders. In a mouse model of chronic migraine, repeated nitroglycerin (NTG, a reliable trigger of migraine in patients) increased the number of CD3+ T cells in the trigeminal ganglia (TG) without altering the number of Treg cells, suggesting a loss of balance between immune activation and suppression. Low-dose interleukin-2 (ld-IL2) treatment preferentially expanded the endogenous Treg cells in peripheral tissues, including the dura and TG. This completely reversed NTG-induced persistent facial skin hyper-sensitivity in both male and female, inbred and outbred mice. Importantly, the basal nociceptive responses were not altered by ld-IL2, and tolerance was not observed. The therapeutic effect of ld-IL2 was abolished by Treg cell depletion and was recapitulated by the adoptive transfer of Treg cells, indicating that endogenous Treg cells mediate the effects of ld-IL2. Ongoing experiments focus on elucidating the molecular mechanisms underlying ld-IL2's beneficial effects. In a mouse model of mild traumatic brain injury (mTBI), ld-IL2 treatment 1 week post-injury not only accelerated the resolution of behavioral sensitization related to acute PTH, but also prevented the development of hyperalgesic priming related to chronic PTH. Moreover, starting ld-IL2 treatment 5 weeks post-

injury effectively reversed mTBI-induced hyperalgesic priming, suggesting that delayed ld-IL2 treatment may be effective for both acute and chronic PTH. At cellular level, ratiometric Ca2+ imaging analysis revealed that repeated NTG and mTBI
significantly increased the number of TG neurons that respond to neuropeptides CGRP and PACAP (calcitonin gene-related peptide and pituitary adenylate cyclase-activating polypeptide). This was completed blocked by ld-IL2 treatment, suggesting that ld-IL2 reverses chronic headache-related behaviors through inhibition of peripheral sensitization. Collectively, the present study identifies Treg cell as a promising target and ld-IL2 as a potential therapy for chronic migraine and PTH with a mechanism of action distinct from the existing treatment options.

Disclosures: J. Zhang: None. **K. Czerpaniak:** None. **Z. Guo:** None. **X. Liu:** None. **J. Unsinger:** None. **R. Hotchkiss:** None. **Z. Qin:** None. **Y. Cao:** None.

Poster

P384. Headache and Migraine: Models and Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P384.10

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS104200

Title: Efficacy of a Dual ENKephalinase Inhibitor in a preclinical stress-induced migraine model

Authors: *H.-R. MEI[1], T. OUIMET[2], H. PORAS[2], G. O. DUSSOR[1];
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Abstract: Migraine is the 2nd most disabling disease worldwide. The current treatments for migraine are triptans, NSAIDs, CGRP signaling inhibitors and antiemetics, but many people with migraine do not get adequate relief from any of the currently-available therapeutics. While opioids show efficacy for migraine, their use could lead to medication overuse headache or transformation from episodic to chronic migraine. To determine whether the endogenous opioid system could potentially be utilized as a migraine therapeutic approach, we tested the efficacy of an Dual ENKephalinase Inhibitor (DENKI) (PL37) in a preclinical model of migraine. We have shown previously that repetitive restraint stress (2 hours per day, 3 consecutive days) induces mechanical hypersensitivity and grimace responses as well as priming to the nitric oxide donor sodium nitroprusside (SNP; 0.1 mg/kg). Facial and hindpaw mechanical thresholds were measured using von Frey filaments and grimace scores were evaluated by observation of mouse facial expressions. We tested PL37 at two different phases of this preclinical model. On day 2 post-stress, either i.v. (10 mg/kg) or p.o. (20 mg/kg) PL37 significantly attenuated the stress-

induced facial and hindpaw hypersensitivity, as well as facial grimace responses, while no effect was observed in the vehicle group. Additionally, both i.v. (10 mg/kg) and p.o. (20 mg/kg) PL37 given one hour prior to SNP (0.1 mg/kg, i.p.) on day 14 post-stress significantly reduced SNP-induced facial and hindpaw hypersensitivity. To test whether opioid receptors mediate these effects, mice were pretreated with the peripherally-restricted opioid antagonist naloxone methiodide (5 mg/kg, s.c.) 30 min prior to PL37, which blocked the effects of this compound. Studies are currently underway to test the efficacy of additional opioid receptor antagonists to further elucidate which opioid receptor mediates the efficacy of PL37 in this model. These data demonstrate that inhibiting enkephalinase in the periphery is effective in treating migraine-like behavior via activation of opioid receptors. Peripheral targeting of endogenous opioid signaling may be an effective novel therapeutic strategy for migraine that lack the negative effects commonly associated with centrally-acting opioids.

Disclosures: H. Mei: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pharmaleads. **T. Ouimet:** A. Employment/Salary (full or part-time):; Pharmaleads. **H. Poras:** A. Employment/Salary (full or part-time):; Pharmaleads. **G.O. Dussor:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pharmaleads.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS118731

Title: Calcitonin gene-related peptide 1 receptor blockade in the amygdala inhibits pain-related behaviors and neuronal activity in a chronic neuropathic pain model

Authors: *P. PRESTO[1], V. NEUGEBAUER[1,2,3];
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Abstract: The amygdala has emerged as a key player in the emotional-affective aspects of pain and pain modulation. The laterocapsular region of the central amygdala nucleus (CeLC) is defined as the “nociceptive amygdala” due to its high content of neurons that process pain-related information. The CeLC is the target of the spino-parabrachio-amygdaloid pain pathway, which is the major source of calcitonin gene-related peptide (CGRP) . Therefore, CGRP acting on CGRP1 receptors is an important molecular marker of the CeLC. Changes in CeLC neurons

have been observed in pain models and synaptic plasticity in the CeLC has been linked to pain-related behaviors. CGRP has been shown to be involved in peripheral and spinal mechanisms and in pain-related synaptic plasticity in the amygdala. However, the role of CGRP-mediated plasticity in the amygdala in neuropathic pain behaviors remains to be determined. Here we tested the hypothesis that the CGRP1 receptor is involved in neuropathic pain-related amygdala plasticity and that blockade of this receptor can inhibit neuropathic pain behaviors. Sensory and affective behaviors were measured in adult chronic neuropathic rats (4 weeks after spinal nerve ligation, SNL). For blockade of the CGRP receptor, a selective CGRP1 receptor antagonist (CGRP 8-37) was administered stereotaxically into the CeLC by microdialysis. Inhibition of the CGRP1 receptor reduced emotional responses to noxious stimuli (audible and ultrasonic vocalizations) and mechanical hypersensitivity (hindlimb withdrawal reflexes). Tonic aversive aspects of pain relief by CGRP 8-37 were detected in the conditioned place preference (CPP) test. In brain slices containing central amygdala corticotropin releasing factor (CeA-CRF) neurons from neuropathic rats, multiphoton imaging showed that CGRP1 receptor blockade reduced calcium signals evoked by electrical stimulation of presumed PB input. Together these findings may suggest that CGRP1 receptors in the CeLC are involved in neuropathic pain-related plasticity and contribute to nociceptive and emotional-affective pain responses. CGRP1 receptors in the amygdala may serve as a therapeutic target for pain relief.

Disclosures: P. Presto: None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS118731
NIH Grant AA027096

Title: Subacute silencing of HMGB1 in the amygdala inhibits pain-related behaviors in a rat model of chronic neuropathic pain

Authors: *R. JUNELL[1], P. PRESTO[1], I. PONOMAREV[1,2], V. NEUGEBAUER[1,2,3];
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. & Therapeut., [3]Garrison Inst. on Aging, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain is a pervasive health care issue that affects a significant portion of the global population each year. An intricate interplay between sensory, cognitive, and emotional-affective dimensions forms the highly complex experience of pain, presenting a challenge to

identifying effective treatment options. Maladaptive neuroplasticity is a key contributor in the transition from acute to chronic pathological pain, and mechanistic investigation of this transition is of particular interest for the potential discovery of new therapeutic strategies. This transition may be associated with neuroimmune mechanisms in the brain, which have yet to be elucidated. The amygdala is a limbic structure that has emerged as a key player in the emotional-affective aspects of pain and pain modulation. The role of neuroimmune signaling in the amygdala in chronic pain states is an important knowledge gap. High motility group box 1 (HMGB1) is a "proinflammatory" molecule that is involved in pain-related crosstalk between neurons and glia in the spinal cord, though its role in the amygdala in pain states has yet to be explored. Here we tested the hypothesis that HMGB1 is involved in pain-related amygdala plasticity and that inhibition of this molecule can reduce neuropathic pain behaviors. Transcriptomic analysis using RNA-Seq and validation using qPCR showed that HMGB1 was upregulated in the central nucleus of the amygdala (CeA) of adult chronic neuropathic rats (4 weeks after spinal nerve ligation, SNL). HMGB1 siRNA (pooled AAV vector) was injected stereotaxically into the right CeA of adult male and female rats as either a pretreatment (2 weeks before) or posttreatment (1 week after) to SNL surgery. Sensory and affective behaviors were measured 4 weeks after SNL. HMGB1 silencing ameliorated anxiety-like behaviors (open field test and elevated plus maze), increased mechanical withdrawal thresholds (von Frey test and tissue compression with calibrated forceps), and reduced emotional-affective responses (audible and ultrasonic vocalizations) in the posttreatment but not pretreatment group. mRNA analysis of right CeA tissues using qPCR after behavioral testing showed upregulation of HMGB1 expression in the pretreatment group compared to sham controls, indicating recovery of HMGB1 at the chronic stage. Together these findings suggest that HMGB1 in the amygdala contributes to the transition from acute to chronic neuropathic pain, and that inhibition of this molecule at a subacute time point may serve as a potential therapeutic target for neuropathic pain relief.

Disclosures: R. Junell: None. **P. Presto:** None. **I. Ponomarev:** None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.03

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS118731

Title: Optogenetic silencing of amygdala CRF neurons inhibits anxiety-like behaviors at the acute stage of a rat neuropathic pain model

Authors: *V. NEUGEBAUER, M. MAZZITELLI, V. YAKHNITSA;
Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The amygdala plays a critical role in the emotional-affective component of pain and in pain modulation. The central nucleus of amygdala (CeA) serves as the major output nucleus of the amygdala. Changes in different subsets of CeA neurons have been observed in pain models and neuroplasticity in the CeA has been linked mechanistically to pain-related behaviors in different pain conditions. While CeA neurons containing corticotropin releasing factor (CRF) have been implicated in affective disorders and pain modulation, their role in different pain conditions and at acute versus chronic stages are not well understood. Here we focused on the effects of optogenetic silencing of CeA-CRF neurons on pain related behaviors in the acute phase of the neuropathic condition. Sensory and affective behaviors were measured in adult neuropathic rats (1 week after spinal nerve ligation, SNL). For optogenetic silencing of CRF neurons, a Cre-inducible viral vector (DIO-AAV) encoding enhanced Natronomonas pharaonis halorhodopsin (eNpHR3.0) was injected stereotaxically into the right CeA of transgenic Crh-Cre rats (original breeding pairs kindly provided by Dr. Robert Messing, UT Austin). Animals were allowed to recover for channel expression. For wireless optical stimulation of eNpHR3.0 expressing CeA-CRF neurons, an LED optic fiber was stereotaxically implanted into the right CeA in order to deliver yellow (590 nm) light in neuropathic rats. Optical silencing of CeA-CRF neurons ameliorated anxiety-like behaviors (elevated-plus maze and open-field tests) but effects on mechanical withdrawal thresholds (von Frey test and tissue compression with calibrated forceps) and emotional-affective responses (vocalizations) did not reach significance in the acute phase of the neuropathic pain model. These findings suggest that CRF neurons in the CeA contribute to certain neuropathic pain associated behaviors at the acute stage.

Disclosures: V. Neugebauer: None. **M. Mazzitelli:** None. **V. Yakhnitsa:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS109255
NIH Grant NS118731
NIH Grant NS106902

Title: Differential pain-related changes in PB- and PAG-projecting amygdala CRF neurons in a neuropathic pain model

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Abstract: The central nucleus of the amygdala (CeA) plays a key role in pain modulation and pain-related emotional-affective aspect of pain. The CeA contains a high level of corticotropin releasing factor (CRF)-expressing neurons that project to wide brain areas including key pain modulatory regions in the brainstem such as the parabrachial area (PB) and periaqueductal gray (PAG). Our recent study revealed that manipulation of CeA-CRF neurons can modulate neuronal activity in the spinal cord and pain-related behaviors. However, functional differences as well as pain-related cellular and synaptic changes in projection-defined CeA-CRF neurons remain to be determined. Here we sought to determine any differences between PB- and PAG-projecting CeA-CRF neurons in a neuropathic pain model using cell-type specific retrograde labelling and slice patch-clamp recording. Retrograde Cre-dependent adeno-associated viral vectors AAVrg-hSyn-DIO-EGFP and AAVrg-FLEX-tdTomato were stereotaxically injected into the PB and PAG, respectively, of transgenic Crh-Cre rats (original breeding pairs kindly provided by Dr. Robert Messing, UT Austin) to identify PB- and PAG-projecting CeA-CRF neurons in brain slices. Whole-cell patch clamp recordings were obtained from retrogradely labeled CeA-CRF neurons in brain slices from sham control and neuropathic rats (1 week after L5 spinal nerve ligation, SNL model). In current clamp mode, neuronal excitability was measured by injecting depolarizing currents. In voltage clamp mode, excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by electrical stimulation of the basolateral amygdala (BLA) or presumed PB input. We found increased excitability in both PB- and PAG-projecting CeA-CRF neurons in the SNL model compared to sham control. The amplitude of EPSCs were increased in projection-undefined CeA-CRF neurons but changed differently in PB- and PAG-projecting CeA-CRF neurons in the SNL model. These results suggest that CeA-CRF neurons undergo differential changes in pain conditions depending on their projection targets.

Disclosures: T. Kiritoshi: None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.05

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS11873

Title: Differential effects of mGluR2 and mGluR3 on the modulation of CeA-CRF containing neurons in an arthritic model of pain

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Abstract: The amygdala is critically involved in the regulation of emotional-affective components of pain and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions and receives nociceptive information via the external lateral parabrachial nucleus (PB). The CeA contains the highest density of extra-hypothalamic corticotropin releasing factor (CRF), which is implicated in several neuropsychiatric disorders. CeA-CRF containing neurons project to other brain regions involved in behaviors and pain. Gi/o-coupled group II metabotropic glutamate receptors (mGluR2 and 3) are expressed in different brain regions, including the amygdala. Their activation can decrease neurotransmitter release and regulate synaptic plasticity, but effects of the modulation of group II mGluR subtypes on CeA-CRF neurons remain to be determined. Here, we address this knowledge gap in an arthritic pain model. Brain slice physiology was performed to determine the effects of a group II mGluR agonist (LY379268 disodium salt), a positive allosteric modulator (PAM) selective for mGluR2 (LY487379 hydrochloride), and the combination of a group II mGluR agonist (LY379268) with a negative allosteric modulator (NAM) selective for mGluR2 (VU6001966) on CeA-CRF neurons. To visualize CRF neurons, a Cre-inducible viral vector expressing red fluorescent protein (mCherry) was injected into the CeA of transgenic Crh-Cre rats (original breeding pairs kindly provided by Dr. Robert Messing, UT Austin). A viral vector coding channelrhodopsin 2 (ChR2) fused to enhanced yellow fluorescent protein under the control of CaMKII promoter was injected into the PB for optical activation of glutamateric inputs to CeA-CRF neurons. Whole-cell patch-clamp recordings in brain slices from normal and arthritic rats (5-6 h postinduction of a kaolin/carrageenan-monoarthritis) were used to investigate neuronal excitability, monosynaptic excitatory postsynaptic currents (EPSCs), and glutamate-driven inhibitory postsynaptic currents (IPSCs) evoked from PB inputs. Application of LY379268 decreased neuronal excitability and EPSCs and IPSCs in the arthritis condition. Application of LY487379 resulted in decreased EPSCs, whereas selective activation of mGluR3 (combination of LY379268 with VU6001966) mimicked the effects of the group II mGluR agonist on the modulation of CeA-CRF neurons in pain. These results suggest that mGluR2 and 3 have different roles in controlling functions and pain-related changes of CRF neurons in the amygdala, providing important insights into the subtype-specific roles, which may help identify appropriate therapeutic strategies for pain management.

Disclosures: M. Mazzitelli: None. **P. Conn:** None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.06

Topic: D.03. Somatosensation – Pain

Support: NIH Grants NS038261, NS106902, NS109255

Title: Opposing effects of kappa and mu opioid receptors on amygdala CRF neuronal activity in a rat neuropathic pain model

Authors: *G. JI[1], T. KIRITOSHI[1], V. YAKHNITSA[1], E. NAVRATILOVA[2], F. PORRECA[2], V. NEUGEBAUER[1];
[1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Pharmacol., Univ. of Arizona Col. of Med.,, Tucson, AZ

Abstract: Chronic pain states have been consistently associated with a loss of the conditioned pain modulation response. Clinical evidence suggests that the amygdala plays a key role in maintaining descending pain facilitation in chronic pain patients. The amygdala is a major site of opioid receptor and the origin of major corticotropin-releasing factor (CRF) containing pathways in the brain, and CRF is a key player in emotional pain-related responses. We have shown that microinjection of a kappa opioid receptor (KOR) agonist into the right central nucleus of the amygdala (CeA) elicits allodynia whereas a mu opioid receptor (MOR) agonist as analgesic effects. We hypothesize that MOR and KOR in the CeA elicit opposing effects on CRF neurons. In this study, we tested effects of KOR and MOR ligands on neuronal activity by measuring synaptically evoked calcium signals in CeA-CRF neurons in rat brain slices with a multiphoton system. To measure calcium signals in CeA-CRF neurons, CRF-cre rats (original breeding pairs kindly provided by Dr. Michael Burman, Univ. of New England) were injected with AAV5-Syn-Flex-GCaMP7s into the right CeA 5-6 weeks before brain slices were obtained to allow viral vector mediated expression of a fluorescent calcium sensor. Sham or SNL surgery was performed one week after viral vector injection. A multiphoton system (Intelligent Imaging Innovations, Inc., 3i, Denver, CO) was used to record calcium transients evoked by electrical stimulation of the dorsomedial fiber tract containing presumed axons from the parabrachial area. The 2-photon (2P) excitation light was generated by a mode-locked Ti:Sapphire laser (920 nm, model Mai-Tai DeepSee, Spectra-Physics, Santa Clara, CA, USA). CeA-CRF neurons that responded to synaptic stimulation were further analyzed. Fluorescent activity and background ($\Delta F/F0$) were analyzed using SlideBook 6 (3i, Denver, CO, USA). Multiphoton imaging of CeA-CRF neurons in SNL rats showed that synaptically evoked calcium signals were decreased by a MOR agonist (DAMGO, 1 μM) or a KOR antagonist (nor-BNI, 1 μM). A KOR agonist (U-69593, 1 μM) alone had no clear effects. Effects of DAMGO were blocked by co-application of U-69593 but enhanced by co-application of nor-BNI. The data show that KOR and MOR ligands elicit opposing effects on synaptically evoked activity of CeA-CRF neurons in a rat neuropathic pain model. These results may suggest that dual action drugs with KOR antagonist/MOR agonist activity could be explored as a novel therapeutic strategy for neuropathic pain.

Disclosures: G. Ji: None. **T. Kiritoshi:** None. **V. Yakhnitsa:** None. **E. Navratilova:** None. **F. Porreca:** None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS106902
NIH Grant NS109255

Title: Kappa opioid receptor blockade reduces pain-like behaviors and restores inhibition of amygdala CRF neurons in a functional pain model in rats

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Abstract: Chronic pain is observed in conditions related to identifiable injury as well as in functional pain syndromes (FPS), including fibromyalgia, irritable bowel syndrome, and migraine, in which the pain cannot be attributed to tissue pathology. The amygdala is an important contributor to pain-related emotional-affective conditions and pain modulation. The central nucleus of amygdala (CeA) is also a major site of opioid receptors. Activation of kappa opioid receptors (KOR) in the CeA has been shown to generate pain-like behaviors, enhance neuropathic pain behaviors, and disinhibit corticotropin releasing factor (CRF) expressing CeA neurons. Similar adverse effects can also be induced by the endogenous KOR agonist-dynorphin. Here we tested the hypothesis that blockade of KOR signaling with a KOR antagonist (nor-binaltorphimine, nor-BNI) in the right CeA inhibits pain-like behaviors by restoring inhibitory control of CRF neurons in the CeA in a rat model of FPS. The FPS model was induced by morphine priming for 7 days (using subcutaneously implanted mini osmotic pumps) followed 2 weeks later by one hour of restraint stress on two consecutive days. The control group received morphine without stress. For behavioral experiments, animals were implanted with microdialysis probes into the right CeA 5 days prior to testing. Brain slice electrophysiology was used to determine the effects of nor-BNI on CeA-CRF neurons. To visualize CRF-expressing neurons in CeA we used CRF-Cre rats (original breeding pairs kindly provided by Dr. Michael Burman, Univ. of New England). AAV5-ChR2-CaMKII-eYFP was injected into the right lateral parabrachial area (LPB) to allow optical activation of glutamatergic synaptic inputs to CeA neurons. Administration of nor-BNI into the right CeA decreased vocalizations evoked by noxious mechanical stimuli and anxiety-like behaviors in elevated plus maze test. Whole-cell patch-clamp recordings of CRF-positive and CRF-negative CeA neurons revealed that in the FPS model, nor-BNI increased optically and electrically evoked inhibitory synaptic currents (IPSCs) from LPB, while decreasing neuronal excitability (frequency-current F-I relationship). Nor-BNI also decreased spontaneous and miniature IPSCs frequency, but not amplitude. The data suggest

that blockade of KOR signaling in a rodent model of FPS reduces pain-like behaviors and restores synaptic inhibition of CRF-CeA neurons through a presynaptic mechanism of action.

Disclosures: Z. Griffin: None. **V.A. Yakhnitsa:** None. **M. Hein:** None. **E. Navratilova:** None. **F. Porreca:** None. **V. Neugebauer:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS106902
NIH Grant NS109255
HIH Grant NS118731

Title: SK2-channel dysfunction in central nucleus of amygdala in chronic neuropathic pain. Evidence for epigenetic modifications

Authors: *V. YAKHNITSA, *V. YAKHNITSA, J. M. THOMPSON, V. NEUGEBAUER, K. PRUITT;
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Abstract: Neuroplasticity in the central nucleus of amygdala (CeA) is linked to pain behaviors, and increased CeA activity contributes to emotional-affective aspects of pain. Small conductance calcium activated potassium (SK2) channels are expressed in the amygdala and inhibit neuronal excitability by mediating afterhyperpolarization, shunting excitatory transmission, and enhancing inhibitory synaptic transmission. Western blotting and RT-PCR reveal decreased levels of SK2 subunit protein and mRNA in the CeA in neuropathic pain, suggesting pretranscriptional SK2 channel downregulation. Chronic pain, stress, and cognitive impairment can induce epigenetic changes such as DNA methylation. Here we test the hypothesis that SK2 gene promoter methylation alters expression of SK2 channels in CeA neurons in chronic neuropathic pain. Audible and ultrasonic vocalizations and mechanical thresholds (von Frey) were measured in neuropathic rats (left L5 spinal nerve ligation model, SNL) and in sham controls 4 weeks after surgery. SNL rats showed increased vocalizations and reduced withdrawal thresholds compared to sham rats. Whole-cell patch-clamp recordings of CeA capsular neurons revealed that in the SNL model apamin-sensitive medium afterhyperpolarizations (mAHP), mediated by SK2 channels, were decreased while neuronal excitability (frequency-current F-I relationship) was increased. Right amygdala tissue was removed and homogenized for DNA extraction. The methylation profile of SK2 gene promoter region was assessed by bisulfite sequencing methods.

Overall, low methylation levels were observed in both SNL and sham rats, however increased methylation of CpG island of SK2 promoter region in the CeA was detected in SNL group. Interestingly, methylated CpG sites identified in the SNL rat CeA samples were different from those seen in the sham rats. A total of 11 CpG sites ranging from position -832 to -383 relative to the transcription start site of SK2 gene were methylated in SNL rats. Only 3 sites at these positions were found to be methylated in the sham rat CeA (-759, -676, and -383). We also found differential DNA methylation within consensus sequences of transcription factor binding sites across the methylated CpGs in SNL as compared to sham group. The data demonstrate loss of SK2 channel function in CeA in neuropathic pain. Methylation changes in the pain model suggest reduced gene expression by epigenetic mechanisms. Nevertheless, further studies are needed to determine the effect of DNA methylation on transcription factor binding to these differentially methylated transcription regulatory regions and their role in modulating the expression of SK2 gene.

Disclosures: V. Yakhnitsa: None. **J.M. Thompson:** None. **V. Neugebauer:** None. **K. Pruitt:** None.

Poster

P387. Descending Modulation Pain: Amygdala

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P387.09

Topic: D.03. Somatosensation – Pain

Support: NIH Grant DE029342
NIH Grant F31NS122489

Title: Dissecting a neural substrate for stress-induced analgesia

Authors: *C. DIAZ[1], B. CHEN[3], F. WANG[3], K. M. FRANKS[2];
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Abstract: Affective modulation of pain is an important mechanism for selecting appropriate behavior given different contexts. As an adaptive mechanism, it can aid an animal in surviving a life-threatening situation. Stress-induced analgesia (SIA) is such a phenomenon in which an affective state of high stress, induced by such stimuli as predator odor, produces analgesia to noxious stimuli. Previous studies of SIA have shown that stressful stimuli increase freezing behavior and decrease nociceptive responses, a readout of analgesia. Furthermore, SIA was shown to critically depend on the central amygdala (CeA). CeA is known to have a multitude of roles in processing emotional responses to pain and fear. We recently described a distinct population of CeA neurons that are activated by general anesthetics (CeA_{GA}). Optogenetically stimulating CeA_{GA} neurons produces robust analgesia[1]. Furthermore, we have found that CeA_{GA}

cells receive strong projections from a region called the amygdala-piriform transition zone (AmPir), and this region is known to be activated by predator odor[2]. Here we show that the predator odor 2MT reduces nociceptive behavior and increases freezing behavior, functioning as a model of SIA. Additionally, we have found that exposure to the predator odor 2MT activates many fear regions in the brain, including CeA, specifically CeA_{GA}, and AmPir, through calcium imaging and cFos expression. Future work will dissect this AmPir-CeA_{GA} circuit and its function in SIA, providing novel insights into the neural circuits mediating the interplay between stress and pain, and advance our understandings of how emotions modulate pain.

Disclosures: C. Diaz: None. **B. Chen:** None. **F. Wang:** None. **K.M. Franks:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.01

Topic: D.03. Somatosensation – Pain

Support: NIMH Grant 1R01MH116203
Simons Foundation Bridge to Independence Award SFARI 388708
Brain Research Foundation Fay/Frank Seed Grant
Salk Women & Science Special Award
Mary K. Chapman Foundation Fellowship
Jesse & Caryl Philips Foundation Fellowship
NIH-NCI CCSG Grant P30 014195

Title: Divergent opioidergic brainstem pathways that mediate pain-breathing interaction

Authors: *S. LIU[1,2], M. YE[1], G. M. PAO[1], S. M. SONG[2], J. JHANG[1], H. JIANG[2], J. KIM[1], S. J. KANG[1], S. HAN[1];
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Abstract: Breathing and pain perception seem to be unrelated critical brain functions, yet they influence each other. Severe pain induces hyperventilation, whereas paced slow breathing alleviates pain perception. However, a neural circuit-based understanding of pain-breathing interaction is lacking. Here we report that *Oprm1* (μ-opioid receptor)-expressing neurons in the lateral parabrachial nucleus (PBL) are crucial for regulating breathing, pain, and their interaction. The PBL^{Oprm1} activity is tightly correlated with the breathing rhythm, and noxious stimuli simultaneously elevate both. Manipulating PBL^{Oprm1} activity collectively modulates breathing rhythm and negative affects, such as pain and anxiety. Furthermore, we find that the association of pain and breathing is mediated by two non-overlapping but reciprocally connected *Oprm1* subpopulations in the core and shell of the PBL, which diverge to the central amygdala and

medullary respiratory center, respectively. These results uncover the role of the parabrachial opioidergic pathways in regulating basal respiratory rhythm and pain-breathing interaction.

Disclosures: S. Liu: None. **M. Ye:** None. **G.M. Pao:** None. **S.M. Song:** None. **J. Jhang:** None. **H. Jiang:** None. **J. Kim:** None. **S.J. Kang:** None. **S. Han:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.02

Topic: D.03. Somatosensation – Pain

Support: NIH R35 #NS097396 (AIB)
Open Philanthropy

Title: Mapping CNS correlates of pain processing, opioid tolerance, and pain processing in the tolerance setting

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Abstract: Tolerance to opioid analgesics is not only a clinical problem that impedes effective pain management, but it may also be a mechanism of sensitization in chronic pain. Previous studies demonstrated that long-term exposure to opioids induces a state of latent sensitization in neurons of the spinal cord and brain, which may manifest as hypersensitivity in the tolerant state and even post withdrawal. This study aims to assess alterations in the patterns of neuronal activity in opioid tolerance and after acute noxious stimulation in morphine-tolerant mice. Using a $Fos^{2A\text{-}iCreER}$ mouse line with the TdTomato Cre reporter Ai14, once stable tolerance has been established, we injected 4-hydroxytamoxifen (4-OHT) in TRAP2 (Targeted Recombination in Active Populations) mice, which then triggers activity-dependent genetic labeling of neurons throughout the brain and spinal cord. Cre-recombination induced by 4-OHT drives TdTomato expression in active cells when the immediate early gene *c-fos* is induced. Activated neurons are thus permanently TdTom+. Groups of male and female adult TRAP2:Ai14 mice were made tolerant with a subcutaneous (s.c.) implant of a 25mg morphine or placebo pellet. Acute morphine analgesia and tolerance to 10mg/kg morphine sulphate injection s.c. was measured using the tail immersion test, before and 3 days after pellet implantation. 4-OHT i.p. was injected 7 days later, so as to capture neuronal populations active in morphine-tolerant mice and pellet-implanted controls. Seven days later the mice received 2% formalin/10µl injection into the hindpaw. Ninety minutes later the mice were euthanized, and tissue processed for Td-tomato and immunoreactive Fos, which marks neurons “active” in the tolerant state and in response to the formalin in the tolerant state, respectively. Whole brain and spinal cord were immunostained and

cleared using iDISCO (Immunolabeling-enabled 3-Dimensional Imaging of Solvent-Cleared Organs) followed by quantification of TdTom+ and Fos+ cells using light-sheet and confocal imaging and ClearMap for analysis of 3D datasets. Preliminary results show unique patterns of activity in response to formalin in tolerant compared to non-tolerant mice, in several areas of the brain involved in pain modulation, notably the periaqueductal gray. Ongoing studies are also comparing the patterns of neuronal activity manifest in the opioid tolerant CNS with patterns generated in different chronic pain conditions, with the eventual goal of mapping anatomical regions and circuits that contribute to opioid- and opioid tolerance-dependent mechanisms of chronic pain development.

Disclosures: S.J. Chhaya: None. **A.I. Basbaum:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.03

Topic: D.03. Somatosensation – Pain

Support: by NIH grant R01DA044481
DoD Neurosensory Research Award W81XWH-15-1-0076
New York Stem Cell Foundation – Robertson Investigator Award
NIH fellowships T32GM089626
T32DA035165
HHMI medical research fellowship

Title: Resolving Delta Opioid Receptor Distribution and Function in Pain Neural Circuits

Authors: *W. MCCALLUM[1], D. WANG[2], A. SHUSTER[2], D. J. BERG[2], S. LOW[2], G. SCHERRER[1,3];
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Abstract: Resolving Delta Opioid Receptor Distribution and Function in Pain Neural Circuits
The delta opioid receptor (DOP) is an attractive drug target to develop novel opioid analgesics with fewer side effects, compared to mu opioid receptor (MOP) agonists such as morphine. However, the mechanisms of action of DOP and MOP within neural circuits remain poorly understood due to the lack of genetic tools to investigate the neurons that express each receptor. To address this issue, we generated knockin mouse lines in which we targeted the *Oprd1* and

Oprm1 genes that encode DOP and MOP to express the DNA recombinases Cre or Flp. Here, we describe the characterization of *Oprd1*^{Cre} mice. To identify CNS neurons with Cre activity in adult mice, we first used intracranial and spinal injections of AAV5-CAG-FLEX-GFP or intravenous (iv) injections of AAVPHP.eB-CAG-FLEX-GFP. To examine *Oprd1* expression in DRG neurons, we made iv injections of AAVPHP.S-CAG-FLEX-GFP to *Oprd1*^{Cre} mice. We also crossed *Oprd1*^{Cre} mice with Ai14 (Rosa26 LSL-tdTomato) and *Oprd1*^{GFP} mice to compare the *Oprd1* expression fate map (*Oprd1* lineage) and *Oprd1* expression pattern in the adult mouse. In the brain, we found that the Cre activity distribution pattern in *Oprd1*^{Cre} mice is consistent with the known expression pattern of DOP in wildtype mice, for example with GFP+ layer II/III and V cortical neurons, basolateral amygdala neurons, and striatal neurons. In the spinal cord, we observed GFP+ neurons throughout the grey matter, including in the ventral horn, and with a particularly high density at the ventral border of lamina II inner, confirming our recent findings (Wang et al., Neuron, 2018). In DRG, these studies revealed the remarkably dynamic expression of DOP through embryonic development, during which considerably more neuronal types express *Oprd1*, including peptidergic C nociceptors that express *Oprm1*, before DOP expression is extinguished in these cells postnatally. Thus, in the adult DRG, Cre activity is largely restricted to mechanosensory neurons, as evidenced by the presence of tdTomato+ and GFP+ large diameter DRG neurons with axons that project both to spinal laminae III-IV and to medullary cuneate and gracile nuclei through the dorsal column pathway. Collectively, our data establish and validate the utility of *Oprd1*^{Cre} mice to investigate and manipulate DOP-expressing neurons, and begin to define the temporal and spatial characteristics of *Oprd1* and *Oprm1* (co)-expression for elucidating the functional organization of the opioid system.

Disclosures: W. McCallum: None. **D. Wang:** None. **A. Shuster:** None. **D.J. Berg:** None. **S. Low:** None. **G. Scherrer:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.04

Topic: D.03. Somatosensation – Pain

Support: NIH DA041560
NIH DA043204
NIH NS076517
NSF CHE-1625735
Camille and Henry Dreyfus Foundation (BL-19-004)
Milwaukee Institute for Drug Discovery
University of Wisconsin-Milwaukee's Shimadzu Laboratory for Advanced and Applied Analytical Chemistry

Title: Assessing the synergistic effects of morphine and MP-III-024 co-administration: enhanced antinociception with reduced side effects

Authors: *A. ANTONIO JUAREZ[1], E. DIDOMENICO[1], S. URIBE[1], C. DONEGAN[1], Y. TAPIA[1], J. CONVISER[1], R. NAMBIAR[1], C. SIDE[1], M. POE[2], D. SHARMAN[2], J. COOK[2], B. D. FISCHER[3], T. M. KECK[1];
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Abstract: Opioid analgesics are critical for acute and chronic pain management, but important side effects—including tolerance, constipation, respiratory depression, and abuse liability—limit their safety and utility. To provide patients with safer analgesic options, it is critically important to identify of new pharmacotherapeutic strategies to treat pain. Activation of μ-opioid receptors (MORs) in central and peripheral nociceptive pathways mediates opioid analgesia and their critical side effects. Antinociception can also be achieved via selective enhancement of GABAergic signaling at ionotropic GABAA receptors. α2 and α3 subunit-containing GABAA receptors (α2/α3GABAA), which are co-expressed with MORs in dorsal horn spinal pathways important to nociceptive transmission, can be selectively targeted with novel imidazodiazepine positive allosteric modulators (PAMs), such as MP-III-024, which produces antinociceptive effects with limited behavioral disruption. MP-III-024 co-administered with morphine produces synergistic antinociceptive and anti-hyperalgesic effects. In this study, we evaluated whether MP-III-024/morphine co-administration produces sub-additive or synergistic effects in behavioral tests sensitive to morphine side effects. Herein we report that co-administration of MP-III-024/morphine at a 0.94/1 ratio (synergistic in models of antinociception) produced sub-additive effects in morphine-induced hyperlocomotion and in measures of behavioral disruption in food-maintained operant responding. Ongoing studies are evaluating the effects of MP-III-024/morphine co-administration on tolerance in the hot plate test and in conditioned place preference. These experiments are the first comprehensive preclinical analyses of a dual MOR-α2/α3GABAA pharmacotherapy strategy which may increase the therapeutic window between desirable opioid analgesic effects and side effects.

Disclosures: A. Antonio Juarez: None. **E. Didomenico:** None. **S. Uribe:** None. **C. Donegan:** None. **Y. Tapia:** None. **J. Conviser:** None. **R. Nambiar:** None. **C. Side:** None. **M. Poe:** None. **D. Sharman:** None. **J. Cook:** None. **B.D. Fischer:** None. **T.M. Keck:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.05

Topic: D.03. Somatosensation – Pain

Support: P50 DA044121

Title: Sex Differences in Vapor Fentanyl Self-administration Outcomes in Mice With Chronic Neuropathic Pain

Authors: *M. CENTENO[1], R. JABAKHANJI[2], J. M. COX[4], D. SURMEIER[5], A. V. APKARIAN[3];
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Abstract: Due to technical difficulties, we have little knowledge regarding the interaction between opioid
dependence and opioid induced pain relief in rodent models of chronic pain. A knowledge gap that
critically impacts on mechanistic studies which would importantly impact on clinical decisions regarding the current opiate epidemic. Here, following methods described by Moussawi et al. 2021,
we constructed a noninvasive mouse self-administration using fentanyl vaping. With this set-up we
could study neuropathic mice regarding pain and opioid consumption (reinforcement, extinction, reinstatement, motivation) over multiple months.
Mice (n=20), 50% female, were operated for spared nerve injury (SNI, 50%) or sham. Three months
later they were exposed to self-administered fentanyl vapor. Initial dose of fentanyl was 5.0 mg/ml, which was decreased to 2.5 mg/ml in later sessions. Daily (2 hour), fixed ratio (FR1,5,10, 5
days/FR) was followed with extinction, reinstatement, and progressive ratio regiments. The procedure was repeated for the lower dose fentanyl. Active and inactive nosepokes (AN, IN), and
vape puff numbers (reward) were monitored. We also quantified constipation by counting poops/vaping
exposure. Tactile allodynia was also monitored once/week, at the termination of a FR sequence.
Mice learned to discriminate AN from IN with first exposure and maintained constant rate of reward
consumption across increasing FRs. They exhibited signs of dependence: high rates of AN during
reinstatement, in early phase during extinction, and high motivation by completing most PR episodes. Main group difference was in female SNI who showed lowest AN across varying FRs and
lowest discrimination ratio (AN-IN)/AN+IN) (2-way ANOVAs, significant interaction by sex and type
of injury, $p<0.05$). Unexpectedly, we also observed that fentanyl vaping induced, besides hyperalgesia on the uninjured paw, complete reversal of tactile allodynia for the SNI paw which persisted for more than a week and was more prominent in female SNI. Constipation was less in

males throughout extinction, indicating higher stress with fentanyl abstinence (2-way ANOVA, significant interaction by sex and type of injury, $p<0.05$).
Fentanyl vaping provides an ideal environment to study opiate exposure in chronic pain. Our results
challenge current concepts and suggest important sex dependence of opiate addiction and opiate pain
relief in chronic pain.

Disclosures: M. Centeno: None. **R. Jabakhanji:** None. **J.M. Cox:** None. **D. Surmeier:** None. **A.V. Apkarian:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.06

Topic: D.03. Somatosensation – Pain

Support: K01 DA042902
Purdue University College of Pharmacy

Title: Adenylyl cyclase 1 involvement in chronic pain and morphine tolerance in mice

Authors: A. DOUCETTE[1], K. JOHNSON[1], A. EDWARDS[1], A. VERDI[1], R. MCFARLAND[1], V. J. WATTS[2], ***A. KLEIN**[1];
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Abstract: Chronic opioid use for pain management leads to tolerance and dosage escalation, potentially increasing substance dependence. Upregulation of adenylyl cyclase activity has been previously implicated in opioid tolerance and opioid induced hyperalgesia and chronic pain. The objective of this study was to examine the potential adenylyl cyclase isoforms involved in these processes in the peripheral and central nervous system. We also tested the hypothesis that a reduction in adenylyl cyclase 1 (AC1) activity would attenuate morphine tolerance and hypersensitivity, and inflammatory pain using murine models. Mice were subjected to twice daily treatments of saline, 15 mg/kg morphine, for 5 days. dorsal root ganglia (DRG) and spinal cord (SC) were collected from mice in each treatment group for Quantitative PCR. mRNA expression of adenylyl cyclase (*Adcy*) 1, 2, 3, 5, 6, 8; Epac 1 (*Rapgef 3*) and Epac 2 (*Rapgef 4*); and PKA subunits A and B (*Prkaca and Prkacb*) were compared to vehicle controls. Morphine tolerance (15mg/kg, sc, 5 days and 10-40mg/kg escalation over 4 days) and opioid-induced hypersenstivity were assessed after intrathecal administration of an AAV9 viral vector encoding a *s*hort-hairpin RNA (shRNA) of *Adcy1* (AAV9-GFP-U6-m-Adcy1-shRNA) and compared to viral controls (AAV9-GFP-U6-m-scrambl-shRNA). Behavioral testing such as open field testing,

rotarod testing, burrowing, thermal and mechanical paw withdrawal latencies were also tested after injection. Systemic administration of an AC1 inhibitor, ST 034307 was also tested during chronic morphine administration. Morphine induced hypersensitivity was attenuated after Adcy1 shRNA or pharmaceutical inhibition of AC1. Loss of Adcy1 activity decreases morphine tolerance and opioid-induced hypersensitivity, which could form the basis for novel therapeutics in the future.

Disclosures: A. Doucette: None. **K. Johnson:** None. **A. Edwards:** None. **A. Verdi:** None. **R. McFarland:** None. **V.J. Watts:** None. **A. Klein:** None.

Poster

P407. Opioids and Pain

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P407.07

Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award #ADHS18-198875
UA Institutional Funds

Title: Inhibition of Spinal Cord Hsp90 Enhances Src Kinase and Protein Kinase C signaling to Increase Opioid Anti-Nociception

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Abstract: Chronic pain remains a huge social and economic burden in the world. With few safe treatment options, many physicians turn to opioid analgesics. These medications are not without serious side effects including respiratory depression and addiction. Ultimately this has led to an opioid addiction crisis, emphasizing the need to find new ways to improve opioid therapy and decrease side effects. One such research area is the signal transduction cascades evoked by opioid receptor activation, which could be modulated to enhance pain management while decreasing side effects. Previously, our lab discovered that the molecular chaperone protein Heat shock protein 90 (Hsp90) plays a key role in mu opioid receptor signal transduction. When inhibiting Hsp90 in the spinal cord through intrathecal (IT) administration of 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) in mice we observed a significant increase in morphine anti-nociception, which could enable an opioid dose-reduction strategy. Two targets, Src Kinase and Protein Kinase C were hypothesized to be upregulated by 17-AAG treatment. CD-1 mice were treated with 0.5 nmol 17-AAG or vehicle IT. After 24 hours, mice were treated with 10 nmol of the Src kinase inhibitor Src-I1 or vehicle IT, followed by 3.2 mg/kg morphine and a tail

flick assay was performed. This was repeated using 1 nmol of the PKC inhibitor GO6983. The enhanced anti-nociceptive effects seen with 17-AAG were completely abolished in the inhibitor groups, suggesting that Hsp90 inhibition enhances Src and PKC signaling to lead to enhanced opioid pain relief. Interestingly, the group that did not receive 17-AAG but did receive Src-I1 also showed a decrease in the anti-nociceptive effects of morphine, suggesting a role for Src in the opioid receptor pathway at baseline. A post-surgical paw incision pain model showed similar results. The PKC activator phorbol myristate acetate (PMA) at a 0.1 nmol dose produced a drastic increase in antinociception. Initial analysis by Western blot shows the two signaling kinases to be upregulated by 17-AAG treatment and suggests a pathway that includes ERK downstream of both. Analysis via immunohistochemistry of mice treated with 17-AAG and the potent mu agonist DAMGO showed significant increases in Src and PKC phosphorylation. Initial co-staining suggests that Src may be upregulated in different cells depending on treatment. CRISPR knockdown of the Src Kinase also blocked enhanced antinociception in tail flick behavior. These findings have helped elucidate two key molecular mechanisms by which Hsp90 regulates opioid signaling and enhances opioid antinociception, creating potential targets to improve opioid treatment.

Disclosures: J. Bowden: None. **J. Streicher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Teleport Pharmaceuticals, LLC, Botanical Results, LLC.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.01

Topic: E.02. Cerebellum

Support: Mcknight Foundation Technology Grant

Title: Anatomical tracing of cerebello-thalamo-cortico-striatal connections

Authors: *J. HENNESSEY[1], C. CHEN[2], I. A. SAVTCHOUK[1];
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Abstract: The cerebellum is crucial for the execution of complex and fine motor behavior, and damage to the cerebellum can lead to severe motor deficits. Perhaps unexpectedly, the cerebellum also has important non-motor-related functions, including contributions to higher cognition and emotional regulation. Damage to the cerebellum in humans can result in Schmahmann Syndrome (Cerebellar Cognitive Affective Syndrome), deficits in social interactions, linguistic processing, executive function, and reward processing, and obsessive-compulsive disorders. It has even been suggested that cerebellar abnormalities are fundamental

to the development of autism spectrum disorders. Although it is of broad clinical and scientific interest to understand the contributions of the cerebellum to non-motor cognitive function, pre-clinical animal models appropriate for studying non-motor aspects of the cerebellar function are rare.The cerebellum is known to be extensively connected to the cerebral cortex via thalamic and pontine nuclei. Various regions of motor and non-motor cerebral cortex send information to the cerebellum, the cerebellum performs computations, and the cerebellum in turn projects disynaptically back to the same regions of cortex, forming a closed loop of information processing. Recent anatomical studies indicate cerebellar interconnection with the cortical-basal ganglia-thalamus loops. While the exact connectivity is not precisely known, motor and non-motor thalamic regions are involved: MD, VA, VL, VM, anterior intralaminar nuclei and centromedial/parafascicular nuclei. It is hypothesized some of these cortical-striatal-cerebellar loops are essential to not only motor cerebellar function, but also to higher-order functions like cognition, emotion, and reward anticipation.To better describe these subcortical connections, we performed anatomical tracing of projections from the deep cerebellar nuclei (DCN) of the cerebellum to motor and non-motor thalamic regions. This is achieved through unilateral stereotaxic injections of an adeno-associated viruses into the DCN of adult female mice, causing an expression of fluorescent markers in infected neurons. Similarly, we used retrograde AAV injections to the thalamus to back-label the DCN efferents. Neuronal connections to thalamic nuclei were subsequently reconstructed via two-photon imaging of 350 µm slices. Projections from the interposed nucleus were of particular interest, as this set of nuclei has previously been shown to be involved in cognitive functions relating to habit formation.

Disclosures: J. Hennessey: None. **C. Chen:** None. **I.A. Savtchouk:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.02

Topic: E.02. Cerebellum

Support: Doctoral scholarship CONACyT 619857
CONACyT grant 220412
CONACyT grants 2022
CONACyT grants 154039
DGAPA-PAPIIT-UNAM grants IA200815, IN226517, IN203420
Moshinsky fellowship to F.T

Title: Requirement of the Cerebellum-Thalamo and Cerebellum-Thalamo-Striatal projection on the execution of a simple or complex trajectory movement

Authors: *R. D. CONTRERAS-LÓPEZ, E. DÍAZ-HERNÁNDEZ, J. O. RAMÍREZ-JARQUÍN, F. TECUAPETLA;
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Abstract: The cerebellum and basal ganglia are implicated in motor control through parallel circuits and recently, through subcortical cerebellar-thalamo-striatal (Cb-Th-Str) communication, although this last scarcely explored. This study aimed to characterize the communication properties of the Cb-Th-Str and its role in motor control. For this purpose, we mapped the thalamic nuclei that project to the dorsal striatum and receives synaptic projections from the cerebellum. We observed that the cerebellum communicates with the dorsal striatum through ventral and intralaminar nuclei. Whole-cell recording showed a high probability (80%) of Cb-Th-Str connection, with no difference in synaptic properties between dorsal lateral (DLS) and dorsal medial (DMS) striatum. Interestingly, when performing whole-cell recordings on CB-Th-DLS projections, which were located in ventral lateral (VL), ventral medial (VM), central medial (CM), central lateral (CL), and parafascicular (Pf) nuclei, our results revealed that the PF was the principal relay for cerebellar projections to DLS. Next, to evaluate the contribution of these communications on motor control, mice were trained to execute a simple trajectory movement (reaching 8 mm), followed by a complex trajectory movement (reaching >4 mm and returning to the start position). Then, we conducted optogenetic silencing of cerebellar-thalamic synapses in the relay nuclei of the thalamus (VL, CL, and Pf) or in the di-synaptic communication (Cb-Th-DLS). In the simple trajectory movement, Cb-VL silencing resulted in longer displacement during the reaching movement, while CL silencing increased movement velocity. Interestingly, Cb-Th-DLS silencing showed no effects. To contrast the di-synaptic Cb-Th-DLS role in the acquisition and late training of a complex trajectory movement, we conducted optogenetic silencing of the aforementioned synapses during the first three days of acquisition of the complex trajectory movement and late in training. The silencing of Cb-Th-DLS communication early and late in the acquisition of the complex trajectory impaired the performance, inducing longer displacements in the reaching movement and incomplete trajectories in the returning movement. Our results suggest that the cerebellum establishes significant disynaptic connections with the striatum through thalamic nuclei. We suggest that the Cb-Th-DLS connection may not be required during the execution of a simple trajectory movement, but it has an important role during the early and late phase of the acquisition of a complex trajectory movement, likely due to the seriation of the movements in the complex trajectory demanded.

Disclosures: R.D. Contreras-López: None. **E. Díaz-Hernández:** None. **J.O. Ramírez-Jarquín:** None. **F. Tecuapetla:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.03

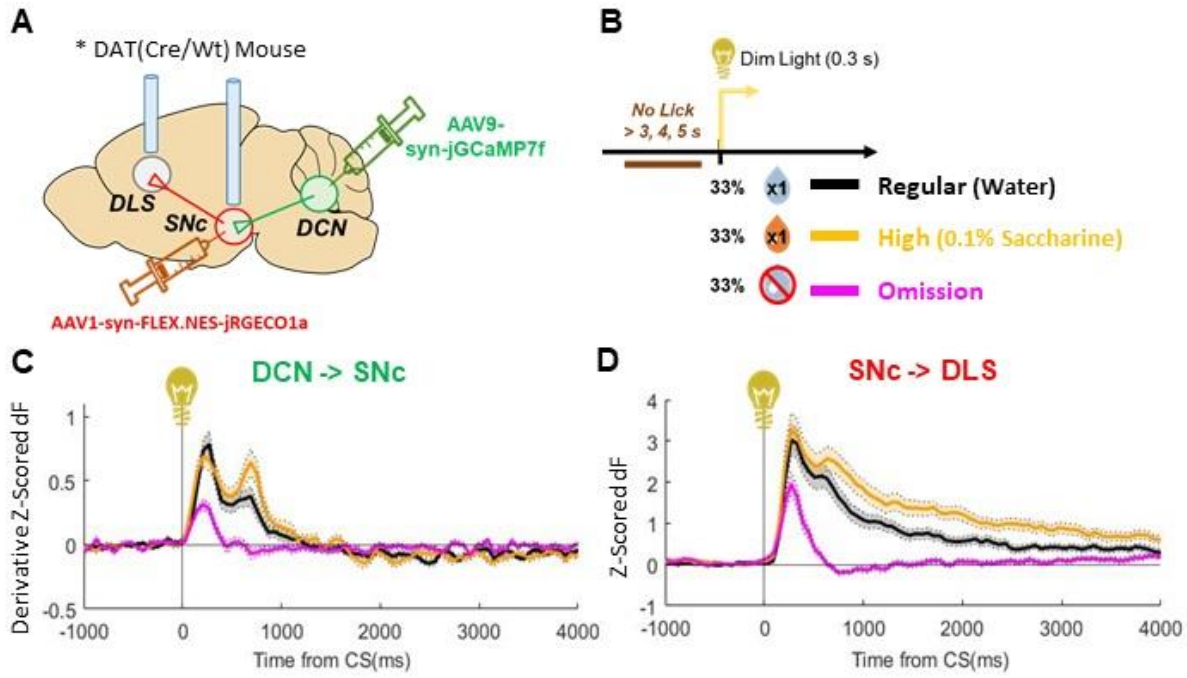
Topic: E.02. Cerebellum

Support: NIDA Grant R01DA044761
NIMH Grant R01MH115604
Grass Fellowship (2018)

Title: Motor and reward-related signaling in the cerebellar-nigral-striatal pathway

Authors: *J. YOSHIDA[1], M. OÑATE[1], V. LOVALLO[1], L. KHATAMI[1], S. G. KEE[1], Y. ISOMURA[2], F. NADIM[3], K. KHODAKHAH[1];
[1]Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Dept. of Physiol. and Cell Biol., Tokyo Med. and Dent. Univ., Bunkyo, Tokyo, Japan; [3]Federated Dept Biol. Sci., New Jersey Inst. of Technol., Newark, NJ

Abstract: The basal ganglia play a central role in voluntary movement and reward-dependent learning. The dopaminergic projections from the substantia nigra pars compacta (SNc) to the dorsal striatum (DS) are an essential pathway in these processes. The cerebellum also contributes to motor control and motor learning, but has been historically considered to work independently of the basal ganglia. We have recently found that the deep cerebellar nuclei (DCN, the main output of the cerebellum), send monosynaptic excitatory projections to the SNc. Additionally, optogenetic stimulation of the DCN-SNc projections induced dopamine release in the striatum, indicating that this pathway could contribute to the basal ganglia function. To understand the functional nature of this connection, using calcium signals as a proxy for neural activity, we recorded this pathway during simple behaviors. Fiber photometry recordings showed that the DCN-SNc projections were activated when the animal initiated a movement such as moving a lever. The DCN-SNc activity was bilateral even with unilateral (e.g. lever) movement. Thus, the DCN-SNc projections have bilateral motor-associated activity. We then used a Pavlovian learning task to examine the DCN-SNc activity in response to randomly-assigned rewards of different quantity and quality. These data indicated that the DCN-SNc activity also has a reward-associated component that is larger with higher reward value. Simultaneous recordings of the DCN-SNc projections and the SNc-DS dopaminergic projections showed a correlation between the activities of both pathways with reward value. However, the SNc-DS dopaminergic population activity was similar in shape to the derivative of the activity of the DCN-SNc pathway, indicating that the dopaminergic neurons may be sensitive to the change in the rate of input from the cerebellum to the SNc. These findings suggest a potentially important role for the cerebellum in how SNc dopaminergic neurons process movement and reward-related learning.



Fiber photometry during Pavlovian Random Reward Task (A) Dual color fiber photometry from DCN (deep cerebellar nucleus) axon in the SNc (substantia nigra pars compacta) and dopaminergic SNc axon in the DLS (dorsolateral striatum). **(B)** Pavlovian Random Reward Task. **(C)** Derivative Z-scored dF of the DCN axon in the SNc and **(D)** Z-scored dF of the SNc axon in the DLS at the CS (conditional stimulus) onset.

Disclosures: J. Yoshida: None. **M. Oñate:** None. **V. Lovallo:** None. **L. Khatami:** None. **S.G. Kee:** None. **Y. Isomura:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.04

Topic: E.02. Cerebellum

Support: EY-015634
EY-017938
EY-020631
EY-017039
EY-019007
Keck, Zegar Family
Dana Foundations

Title: Mid-lateral cerebellar complex spikes encode multiple independent reward-related signals during reinforcement learning

Authors: *N. SENDHILNATHAN[1], A. E. IPATA[1], M. E. GOLDBERG[2];
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Abstract: Although the cerebellum has been implicated in simple reward-based learning recently, the role of complex spikes (CS) and simple spikes (SS), their interaction and their relationship to complex reinforcement learning and decision making is still unclear. Here we show that in a context where a monkey learned to make novel visuomotor associations, classifying CS responses based on their SS properties revealed distinct cell-type specific encoding of the probability of failure after the stimulus onset and the monkey's decision. In a different context, CS from the same cerebellar area also responded in a cell-type and learning independent manner to the stimulus that signaled the beginning of the trial. Both types of CS signals were independent of changes in any motor kinematics and were unlikely to instruct the concurrent SS activity through an error based mechanism, suggesting the presence of context dependent, flexible, multiple independent channels of neural encoding by CS and SS. This diversity in neural information encoding in the mid-lateral cerebellum, depending on the context and learning state, is well suited to promote exploration and acquisition of wide range of cognitive behaviors that entail flexible stimulus-action-reward relationships but not necessarily motor learning.

Disclosures: N. Sendhilnathan: None. **A.E. Ipata:** None. **M.E. Goldberg:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.05

Topic: E.02. Cerebellum

Support: K99NS110978
5R01MH122570-02

Title: Direct Purkinje cell connections onto parabrachial neurons allow the cerebellum to influence multiple forebrain regions and bidirectionally control reward

Authors: *C. CHEN, L. NEWMAN, A. STARK, K. BOND, W. REGEHR;
Harvard Med. Sch., Boston, MA

Abstract: In addition to its well-known contributions to motor control and motor learning, the cerebellum is involved in executive function, emotional regulation, anxiety, and language. Here we investigate how the cerebellum could regulate these processes. We find that suppressing

cerebellar Purkinje cell (PC) firing excites regions implicated in such functions, including the amygdala, the basal forebrain, and the septum. Retrograde tracing experiments revealed that the classic cerebellar outputs from the deep cerebellar nuclei (DCN) do not project to these regions, but that the parabrachial nucleus (PBN) does. We find that PCs directly and tonically inhibit neurons in the PBN, and that suppressing PC firing elevates PBN activity. Using selective suppression of ongoing PC to PBN inhibition, c-Fos labelling, and trans-synaptic tracing we show that PCs inhibit PBN neurons that project to widely to many forebrain regions. We find that suppressing PC-PBN is aversive, in contrast to the well-established rewarding PC-DCN pathway. We conclude that the PC to PBN pathway is an important but underappreciated output pathway that could allow the cerebellum to influence many nonmotor behaviors.

Disclosures: C. Chen: None. **L. Newman:** None. **A. Stark:** None. **K. Bond:** None. **W. Regehr:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.06

Topic: E.02. Cerebellum

Support: ASU start-up

Title: Developmental contribution of dentate nuclei in flexible cognitive behavior

Authors: *T. LYLE, S. SHIPLEY, J. ESPINOZA, J. VERPEUT;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: While the neocortex is predominantly studied for autism pathology, recent evidence has identified decreased connectivity between deep cerebellar nuclei (DCN) and distal cortical regions (e.g., frontal, parietal) associated with theory of mind networks. For clinical interventions to attenuate higher-order (e.g., flexible cognitive behavior) deficits found in autism, additional research identifying neural structure function and influence on neuronal circuits during development is required. Unfortunately, little research has studied the relation between aberrant DCN circuity and cognitive behavior. We have hypothesized that typical maturation of the cerebello-neocortical circuit is required to relay input into distal neocortical regions for cognitive behaviors.
Previously, manipulation of Purkinje cells using chemogenetics, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) has elucidated cerebellar influence on cognitive behavior. Specifically, bilateral inhibition of crus I Purkinje cells (PC) has been shown to impair choice accuracy during an evidence-accumulation decision task and reversal ability in a water Y-maze task. The cerebellum may influence cognition through bidirectional loops between

cognitive regions (e.g., prelimbic and infralimbic cortex) and cerebellar crus I/II, which have been traced using neurotropic viruses. To further this data, we seek to understand the cerebello-thalamo-cortical circuit by analyzing the influence of dentate nuclei during flexible cognitive behavior.
Our objective is to analyze DCN influence on flexible cognitive behavior and neural structure in juvenile mice. PCs project to distal forebrain regions through the DCN, which form dense perineuronal nets at the end of critical periods, suggesting a possible influential neural circuit in early life. To investigate associative functions of crus I projecting PCs to the dentate nucleus, we will use the Gi-coupled hM4Di retro-DREADD (rAAV2-hSyn-DIO-hm4Di(Gi)-mCherry) to drive expression in PC specific, Pcp2 cre mice. Animals will have access to the DREADD ligand clozapine-N-oxide (CNO; 10 mg/kg) in drinking water from postnatal day 21-35. Resulting cognitive behavior will be analyzed using a touch screen pairwise visual discrimination task. Flexible cognition will be tested by reversing the correct stimuli. Post-sacrifice, neural structure in neocortical regions receiving input from the dentate nucleus will be analyzed for dendritic complexity, spine density, and maturation. Using this paradigm, we seek to identify dentate nuclei influence on distal neocortical circuits and flexible cognitive behavior.

Disclosures: T. Lyle: None. **S. Shipley:** None. **J. Espinoza:** None. **J. Verpeut:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.07

Topic: E.02. Cerebellum

Support: NIH grant R15NS112964

Title: Social memory deficit gated by dysregulation of the cerebellar vermis

Authors: O. CHAO[1], S. PATHAK[1], H. ZHANG[1], G. J. AUGUSTINE[2], J. M. CHRISTIE[3], H. TANIGUCHI[4], ***Y.-M. YANG**[1];
[1]Univ. of Minnesota Med. Sch., Duluth, MN; [2]Lee Kong Chian Sch. of Med., Singapore, Singapore; [3]Synapse Physiol. Group, [4]Max Planck Florida Inst., Jupiter, FL

Abstract: Social recognition memory is a key determinant of social behavior. It integrates cognition into social interaction to distinguish between familiar and novel conspecifics. The cerebellum emerges as an important contributor to social functions. For example, reduced activity of Purkinje cells (PCs), in part due to over-inhibition from molecular layer interneurons (MLIs), is a common cerebellar phenotype in various mouse models of autism. Yet, how the cerebellum transforms the abnormal local activity to the global deficit in social behavior remains elusive. To this end, we selectively increase the excitability of MLIs to suppress PC firing with

chemo- and optogenetic approaches in the central vermis of the mouse cerebellum. The chemogenetic perturbation impairs animals' social recognition memory without affecting their sociability, anxiety, motor coordination or object recognition. The optogenetic interference during distinct phases of the social recognition test highlights the cerebellar engagement in the retrieval, but not encoding, of social information. Functional mapping with an immediate early gene product (c-Fos) following the social recognition test reveals a significant reduction in the interregional correlations and a network shift from medial prefrontal cortex (mPFC) and hippocampus-centered to amygdala-centered modules by the chemogenetic manipulation. The social recognition task-induced expression of phosphorylated TrkB receptor for brain-derived neurotrophic factor (BDNF) in the mPFC and hippocampus is inhibited by the optogenetic stimulation, indicating a potential role of BDNF signaling in the brain network shift. Transsynaptic neuronal tracing illustrates hierarchical projections from the central cerebellum to the sub/cortical structures. Collectively, our results suggest that the cerebellum organizes the neural matrix required for social memory.

Disclosures: O. Chao: None. **S. Pathak:** None. **H. Zhang:** None. **G.J. Augustine:** None. **J.M. Christie:** None. **H. Taniguchi:** None. **Y. Yang:** None.

Poster

P513. Cerebellum: Long Range Connections II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P513.08

Topic: E.02. Cerebellum

Title: Effects of rodent optogenetic theta burst stimulation of the lateral cerebellar nucleus on prelimbic cortex physiology

Authors: *P. ABBOTT[1], K. L. PARKER[2];
[2]Dept. of Psychiatry, [1]Univ. of Iowa, Iowa City, IA

Abstract: Evidence continues to accumulate suggesting the cerebellum’s involvement in non-motor processing. Through wide-reaching polysynaptic connections the cerebellum can influence regions of the cerebral cortex that are involved in cognitive processes. Because of this, the cerebellum is being studied as a therapeutic node for treating cognitive dysfunction in disorders such as schizophrenia, autism spectrum disorder, and major depression. Intermittent theta burst stimulation (iTBS) in particular has been delivered to the cerebellum of healthy and psychiatric patients to influence cognitive functioning. One brain region known to be involved in cognition that is likely manipulated by cerebellar stimulation is the medial frontal cortex (MFC). Literature has shown that evoked cerebellar activity can produce observable changes in the rodent prelimbic cortex (PrL), a subregion of the MFC, across a range of physiological measures including electrophysiology and neurotransmitter detection. However, little is known about

plasticity effects in the PrL after theta burst stimulation of the cerebellum. In this ongoing work, the lateral cerebellar nucleus (LCN) of rats is stimulated using optogenetic theta burst stimulation to focus on measures of electrophysiology and dendritic properties. Multi-site local field potential (LFP) recordings were performed in the rat brain during theta burst stimulation, with an optrode placed in the LCN for simultaneous recording and stimulation. After 10 sessions of intermittent theta burst stimulation, brains were collected for dendritic spine analysis in the prelimbic cortex. It is hypothesized that LCN stimulation will result in changes to both LCN and PrL LFP dynamics and lead to altered quantities and morphological qualities of dendritic spines in the PrL. Results are pending and will have important implications for the administration of cerebellar theta burst stimulation in the clinic as a treatment of brain disorders that present with frontal cortical dysfunction. Knowledge of the mechanisms of plasticity underlying brain stimulation remains an important research avenue in order to deliver the most optimal symptom-focused treatment of brain disorders.

Disclosures: P. Abbott: None. **K.L. Parker:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.01

Topic: E.03. Basal Ganglia

Title: The role of Nurr1 in the circadian fluctuations in the number of tyrosine hydroxylase expressing midbrain dopamine neurons

Authors: *J. B. EELLS, H. S. PARTINGTON, J. M. TRUE;
Anat. & Cell Biol., East Carolina Univ. Sch. of Med., Greenville, NC

Abstract: Alterations in dopamine neurotransmission from midbrain dopamine neurons contribute to symptoms of addiction, depression, schizophrenia, and Parkinson's disease. All of these diseases have a strong circadian component and previous data has also observed fluctuations in dopamine neurotransmission across the circadian cycle, including the number of neurons actively expressing tyrosine hydroxylase (TH). The current study investigated how deficits in Nurr1, an essential transcription factor for expression of the dopamine phenotype and expression of TH during development, affect the circadian fluctuations in TH expression. Additionally, we describe a transgenic mouse model with Cre recombinase expression under control of dopamine transporter (DAT) and yellow fluorescent protein (YFP) Cre reporter which, when combined with immunofluorescence, can be used to differentiate between "current" DA neurons (TH^{+}/ YFP^{+}) and "former" dopamine neurons (TH^{neg}/YFP^{+}). The Nurr1 wild-type (+/+) mice showed circadian cycling of dopamine neuron numbers, with a significantly greater number (~2-fold increase) of dopamine neurons in both the substantia nigra pars compacta and ventral

tegmental area at circadian time (CT) 0 (when lights come on) than CT12 (lights go out). In contrast, the Nurr1-null heterozygous (+/-) mice did not show a circadian cycling of the dopamine phenotype, as no differences in the numbers dopamine neurons were observed between CT0 and CT12. Additionally, the +/- mice had significantly fewer dopamine neurons at CT0 but not at CT12 as compared to +/+ mice. Using the DAT cre/YFP reporter mice, we found, at birth, ~95% of all YFP^+ neurons were TH^+. Comparisons, at CT0 and CT12 across development, found no difference at 21 days of age (CT0: 88.07 ± 1.95% and ZT12: 86.79% ± 7.75%), but a significant difference at 35 days (CT0: 89.36 ± 0.25% and ZT12: 75.0 ± 5.26%) and 60 days of age (CT0: 87.15 ± 4.1% and CT12: 75.08 ± 3.70%). These data demonstrate the capability of visualizing "current" and "former" dopamine neurons and show an essential role for Nurr1 in regulating the number of "current" dopamine neurons across the circadian cycle. Since aging, stress, toxins, and inflammation can reduce Nurr1 expression, these perturbations could have a similar effect on the circadian cycling of the dopamine phenotype and potentially contribute to neurologic and neuropsychiatric symptoms associated with dopamine neurotransmission.

Disclosures: J.B. Eells: None. **H.S. Partington:** None. **J.M. True:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.02

Topic: E.03. Basal Ganglia

Title: Nigral GPR55 receptors stimulate motor behavior through modulation of GABA release.

Authors: J. AVALOS-FUENTES[1], F. PAZ-BERMÚDEZ[1], *B. FLORAN[2];
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Abstract: Previously we reported that orphan GPR55 receptors stimulate [^{3}H]-GABA release at striato-nigral terminals. Here we study if intra-nigral activation of GPR55 with their endogenous agonist lysophosphatidylinositol (LPI) stimulates motor behavior through stimulation of GABA release and activation of GABA A postsynaptic receptors. LPI (20 µM) induced contralateral turning when intra-nigrally injected. The gradual increase has a maximum of 8±1 turns/min at 10 min after injection. A previous ten min injection of CID 16020046 (10 µM), a GPR55 antagonist, prevents turning induced by LPI. To test if the effect of LPI was mediated by indirect activation of presynaptic striato-nigral D1 receptors, we blockade them by previously injecting the D1 antagonist SCH 23390 (1µM), which itself did not induce turning either did not modify LPI induced circling remaining at 10 min at 8±2 turns/min. Finally, to test if the effect of LPI was mediated by modulation of GABA release, we blockade GABA A postsynaptic receptors by

intranigral injection of bicuculline (15 µgrs/µl). Bicuculline does not modify motor behavior; however, it prevents almost totally turning elicited by LPI. These data indicate that stimulation of nigral presynaptic GPR55 receptors stimulates motor behavior through modulation of GABA release.

Disclosures: J. Avalos-Fuentes: None. **F. Paz-Bermúdez:** None. **B. Floran:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.03

Topic: E.03. Basal Ganglia

Support: NIH Grant DP2NS105553
Charles A. Dana Foundation
Marlene & Paolo Fresco Institute
NYU MSTP Vilcek Scholars

Title: Striatal acetylcholine and dopamine fluctuations maintain a constant temporal relationship in vivo

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Abstract: It is widely believed that there exists a steady state, basal tone of neuromodulators like acetylcholine (ACh) and dopamine (DA) that is periodically interrupted by phasic increases or decreases signaling salient events that need to be attended to, acted on or learned from. Here we employ a combination of techniques to simultaneously monitor cholinergic interneuron activity in vivo, as well as ACh and DA levels with dual color fiber photometry. We show that extracellular ACh levels in the dorsal striatum of mice constantly undergo large fluctuations several times a second, even during quiet wakefulness. We show that these fluctuations are driven by coordinated spiking of cholinergic interneurons. These fluctuations grow larger in amplitude during movement, in phase with acceleration, and are comparable in time course and magnitude to phasic ACh responses typically associated with reward. Importantly, these ACh fluctuations maintain a specific temporal relationship to striatal DA levels across behavioral contexts. These findings question the existence of a basal tone for ACh and DA, and suggest that phasic increases and decreases in ACh and DA are not uniquely associated with reward and salient behavioral events. Rather, our data indicate that striatal DA and ACh signaling is consistently and intrinsically structured, forcing a reconsideration of our understanding of modulation of neural activity and behavior by ACh and DA.

Disclosures: A.C. Krok: None. **P. Mistry:** None. **Y. Li:** None. **N.X. Tritsch:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.04

Topic: E.03. Basal Ganglia

Support: R01NS104944

Title: Acute dopamine modulation of PKA in the dorsal striatum during locomotion

Authors: *L. MA[1], O. P. JAIDAR[2], J. B. DING[3], T. MAO[4], H. ZHONG[5];
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Abstract: Acute dopamine modulation of PKA in the dorsal striatum during locomotion
Lei Ma[1], Omar Pedro Jaidar Benavides[2], Jun Ding[2], Tianyi Mao[1], and Haining Zhong[1,*]1. Vollum Institute, Oregon Health & Science University, Portland, Oregon, 972392. Department of Neurosurgery and Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, CA, 94305
Dopaminergic inputs to the dorsal striatum play essential roles for locomotion. The effects of dopamine are thought to be mediated by its modulation of medium spiny neuron (MSN) excitability through pathways that involve cAMP/protein kinase A (PKA). However, little is known about the dynamics of PKA signaling in MSNs following dopamine activation *in vivo*. The precise contribution of PKA to dopamine related physiological functions also remains to be determined.
These questions have not been addressed in part due to the lack of effective techniques to monitor real-time PKA activity in neuronal populations in behaving mice. By combining an optimized genetically-encoded PKA sensor, tAKARα, with two-photon fluorescence lifetime imaging microscopy (2pFLIM), we have recently established PKA activity imaging with single-cell resolution in the cortex of head-fixed behaving mice. By performing 2pFLIM imaging of tAKARα via a gradient-index (GRIN) lens, we now extend such PKA imaging capability to the striatum, and have successfully observed PKA activity in striatal neurons at subcellular resolutions in defined neuronal types during locomotion.
To determine how dopamine modulates PKA activity in MSNs in the direct and indirect pathways (dMSNs and iMSNs, respectively), we performed PKA imaging in these neuronal types in response to optogenetic stimulation of dopaminergic axons in the dorsal striatum. We found that optogenetic release of dopamine in the dorsal striatum leads to cell type-specific dopamine responses. Our ongoing work will further assess whether PKA activity is regulated by

locomotion and aim to provide mechanistic understanding of how PKA activity affects neuronal functions and animal behavioral outcomes.

Disclosures: L. ma: None. **O.P. Jaidar:** None. **J.B. Ding:** None. **T. Mao:** None. **H. Zhong:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.05

Topic: E.03. Basal Ganglia

Support: NSF IOS 1655365

Title: Synaptic mechanisms underlying the regulation of striatal excitability by the neuronal glutamate transporter EAAC1

Authors: *M. A. PETROCCIONE, L. D'BRANT, N. AFFINNIH, H. CHESBRO, S. ZAHID, A. SCIMEMI;
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Abstract: Non-functional mutations of the gene encoding the neuronal glutamate transporter EAAT3 (EAAC1 in mice) are associated with obsessive compulsive disorder (OCD). This neuropsychiatric disorder is associated with hyperactivity in the striatum, which is one of the brain regions where EAAC1 is most abundantly expressed. While EAAC1 is expressed at both glutamatergic and GABAergic synapses in other brain regions, the role of EAAC1 at striatal synapses is still poorly understood. Our lab has previously shown that EAAC1 increases the expression of D1-dopamine receptors in striatal medium spiny neurons (MSNs) by limiting the activation of group I metabotropic glutamate receptors (mGluRI). By doing so, it limits the execution of stereotyped grooming behaviors. Here, we show that EAAC1 limits dendritic length, spine diameter and synaptic inhibition only in D1-MSNs. The frequency and amplitude of miniature EPSCs are increased in D1-MSNs of $EAAC1^{-/-}$ mice, as is the quantal size of GABAergic miniature IPSCs. By using optogenetics, we show that only a subset of inhibitory synapses are responsible for these effects. NEURON compartmental models show that these effects allow EAAC1 to reduce the offset and gain of the firing output of D1-MSNs. Together, these findings highlight the importance of EAAC1 in controlling synaptic transmission in the striatum, with potential long-range implications for understanding the synaptic and circuit basis of OCD.

Disclosures: M.A. Petroccione: None. **L. D'Brant:** None. **N. Affinnih:** None. **H. Chesbro:** None. **S. Zahid:** None. **A. Scimemi:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.06

Topic: E.03. Basal Ganglia

Support: Canadian Institutes of Health Research (MOP 136778)

Title: VMAT2 availability in Parkinson's disease with rapid eye movement sleep behaviour disorder

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Abstract: Objective: To use [^{11}C]DTBZ PET imaging to explore vesicular monoamine transporter 2 (VMAT2) availability in Parkinson's disease (PD) with and without rapid eye movement sleep behaviour disorder (RBD). **Background:** RBD is a condition marked by the lack of normal skeletal muscle atonia during REM sleep, resulting in dream enacting behaviours. RBD is one of the strong prodromal clinical predictors of PD. Molecular imaging evidence shows that PD with RBD (PD-RBD+) show lower dopamine transporter activity within the caudate and putamen compared to PD without RBD (PD-RBD-). However, the characterization of the VMAT2, an index of nigrostriatal dopamine innervation, has been rarely explored in PD patients with RBD. **Design/Methods:** We enrolled 15 PD-RBD+, 15 PD-RBD- and 15 age matched healthy controls (HC) for the [^{11}C]DTBZ PET imaging study. This technique measures VMAT2 availability within striatal regions of interest (ROI). Mixed effect model was used to compare the radioligand binding of VMAT2 between the three groups for each striatal ROI, while co-varying for sex, cognitive and depression scores. The fixed factors in this model were group and ROI hemisphere (i.e., left vs. right ROI), while participants were kept as a random factor. Significant level was set at $p<0.05$ (Bonferroni corrected). **Results:** Significant main effect was observed within the caudate, putamen, ventral striatum, external globus pallidus, internal globus pallidus, and substantia nigra. Specifically, we observed that both PD-RBD+ and PD-RBD- group had lower VMAT2 availability compared to HC. **Conclusions:** Our findings reveal that both PD patient subgroups have lower VMAT2 availability relative to HC—which reflects denervation within the nigrostriatal pathway. There is limited indication that VMAT2 contributes differently to PD patients with RBD relative to PD patients without RBD. Future studies are encouraged to explore other underlying neural mechanisms contributing to RBD in PD patients.

Disclosures: M. Valli: None. **S. Cho:** None. **C. Uribe:** None. **M. Masellis:** None. **R. Chen:** None. **A. Mihaescu:** None. **A. Strafella:** None.

Poster

P516. Transmitters and Neuromodulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P516.07

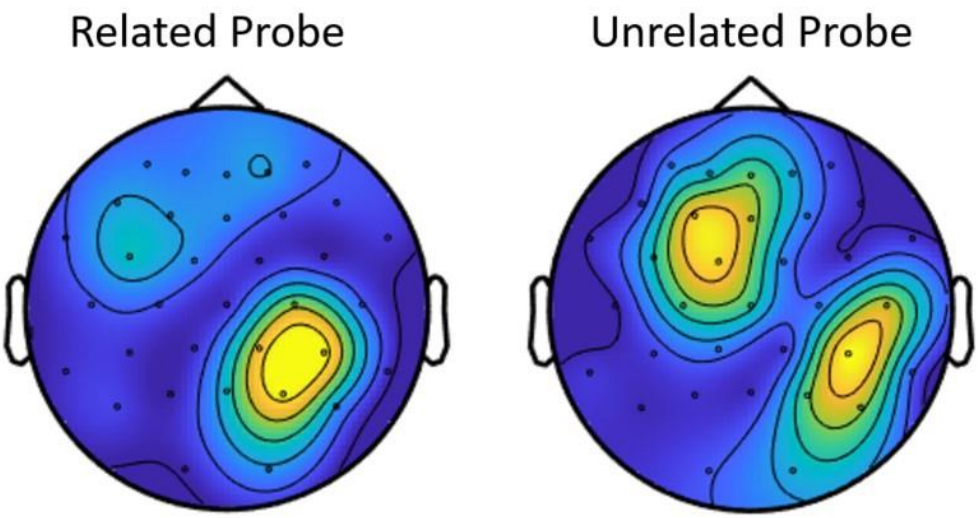
Topic: H.11. Language

Support: NIH 1UH3NS100543
NIH K23NS091344

Title: Cerebellar depth recordings during semantic priming

Authors: *D. P. FLODEN[1], R. GOPALAKRISHNAN[1], A. SRIVASTAVA[2], J. BIARS[1], M. SCHROEDEL[2], K. B. BAKER[2], A. G. MACHADO[1];
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Abstract: The cerebellum's theoretical role in cognitive processes such as language is based on indirect evidence of deficits in patients with cerebellar damage or non-invasive stimulation studies. Here, we present electrophysiological activity recorded from the scalp and cerebellar dentate nucleus (DN) during a semantic priming task. Recordings were obtained in eight patients (mean age 58 ± 8.2; 6 males) enrolled in a clinical trial of DN Deep Brain Stimulation (DBS) to treat upper extremity motor impairments following middle cerebral artery stroke (3 left hemisphere, 5 right hemisphere). Patients viewed 3 to 6 blocks of 100 serially-presented prime-probe pairs in a low-expectancy condition (19% related prime-probe words) followed by a high expectancy condition (73% related prime-probe words). Continuous activity was recorded from 36 scalp electrodes and 8 DBS contacts embedded in the contra-lesional DN. Event-related potentials time locked to onset of the probe word showed the classic N400 component at central-parietal sites that was larger for unrelated pairs relative to related pairs, indicative of greater semantic processing requirements for unprimed words. Time frequency analysis demonstrated increased gamma power (35-45hz) in the same time window after onset of related compared to unrelated probes, consistent with prior studies in neurologically normal adults. Coherence between DN and the contralateral parietal region following onset of related and unrelated probe words was evident in both the high and low expectancy conditions in the 8-15 Hz range. In contrast, coherence between DN and ipsilateral frontal regions in most patients was more specific to unrelated probes in the high expectancy condition during the early semantic processing window of 200-400ms post-onset. This may represent cross-network mismatch between semantic content and predictive models generated by the cerebellum. To our knowledge, these data represent the first evidence of cognitive task-related activity recorded directly from the human cerebellum.



Coherence between left DN and Scalp in High expectancy condition from one subject. Coherence between DN and ipsilateral Frontal areas evident during Unrelated probe.

Disclosures: D.P. Floden: None. **R. Gopalakrishnan:** None. **A. Srivastava:** None. **J. Biars:** None. **M. Schroedel:** None. **K.B. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy Inc. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy Inc. **A.G. Machado:** 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.; Enspire DBS Therapy Inc provides funds to the institution, Boston Scientific Intrepid DBS provides funds to the institution. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy Inc, Cardionomics. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy Inc, Boston Scientific, Abbott.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.01

Topic: E.05. Brain-Machine Interface

Support: Swiss National Science Foundation, DOC-Mobility P1FRP3_188027
NINDS R01NS088184

Title: Amplitude modulated high-frequency stimulation elicits asynchronous axonal spiking activity

Authors: *B. BARRA[1], R. KUMAR[2], C. GOPINATH[1], R. GAUNT[1], M. CAPOGROSSO[1], L. E. FISHER[1];
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Abstract: Regaining sensory feedback is a priority for people with limb amputation, and electrical stimulation of the peripheral nerves can reproduce sensory percepts that feel like they emanate from the prosthetic limb. However, subjects often describe these sensations as unnatural. Traditional electrical stimulation paradigms activate neural structures using charge-balanced rectangular pulses at frequencies lower than 100 Hz. This simplistic approach elicits synchronous responses in all recruited fibers. It has recently been hypothesized that unnatural synchronicity in peripheral neural activity is responsible for several negative effects of electrical stimulation, such as the production of unnatural sensory percepts in amputees (e.g., tingling), and muscle fatigue during functional electrical stimulation in people with spinal cord injury or stroke. Here, we propose a non-conventional stimulation paradigm that consists of an amplitude-modulated, high-frequency (AM-HF) sinusoidal stimulation waveform. We combined computational and experimental approaches to show that AM-HF stimulation produces asynchronous firing in populations of nerve fibers. First, we modeled a set of parallel multicompartmental axons derived from the standard Hodgkin-Huxley formalism and we inserted them in an infinite homogeneous space. All axons were contained within a space of 100 um2 and two ideal current sources were oriented parallel to the axons and placed at 1 mm from the center of the bundle. We computed the responses of the modeled axons to stimulation using the NEURON simulation environment. Our computational model highlighted that the AM-HF waveform induced pseudo-stochastic firing in each axon. Moreover, we found that different axons fired asynchronously during AM-HF stimulation and that the firing rate was modulated by stimulation parameters. Second, we validated the computational results by delivering AM-HF stimulation in an anesthetized cat animal model. We stimulated with AM-HF through a multi-contact cuff (Ardiem Medical) wrapped around the sciatic nerve, while recording with 2 micro-electrode arrays (Blackrock Microsystems, Utah) implanted in the L6 and L7 dorsal root ganglia. Our experimental results corroborated the findings of our computational study: the recorded action potentials mostly occurred asynchronously and were robustly modulated by stimulation parameters. These results represent an important step towards the development of biomimetic stimulation strategies that could be applied in several clinical applications such as for the restoration of sensory feedback.

Disclosures: B. Barra: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Pittsburgh. **R. Kumar:** None. **C. Gopinath:** None. **R. Gaunt:** None. **M. Capogrosso:** None. **L.E. Fisher:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.02

Topic: E.05. Brain-Machine Interface

Support: UH3NS100541

Title: Comparison of coordination in residual limb muscle reflexes and sensory percepts evoked by epidural stimulation of the lumbosacral spinal cord in trans-tibial amputees

Authors: *D. SARMA[1], B. BARRA[2], A. C. NANIVADEKAR[3], B. PETERSEN[3], R. BOSE[3], E. HELM[2], M. BONINGER[2], M. CAPOGROSSO[4], L. E. FISHER[2], D. J. WEBER[1];
[1]Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [2]Physical Med. and Rehabil., [3]Bioengineering, [4]Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In the lower limbs, bilaterally coordinated spinal reflexes are evoked by sensory inputs from the foot and ankle. These reflexes largely mediate the transitions between phases of gait as well as managing stability after unexpected perturbations like slips and trips. Without these sensory inputs, lower-limb amputees experience approximately double the annual incidence of falls compared to able-bodied individuals. Lumbar epidural spinal cord stimulation (eSCS) is an intervention that can modify dysregulated lower-limb sensorimotor function and augment residual motor capacity. In two below knee amputees, eSCS was delivered through 2-3 percutaneously implanted (<30 days) commercial 16-electrode leads (Boston Scientific) over the L1-L3 spinal cord and dorsal roots. eSCS was delivered at pulse widths of 0.2 to 1ms at 1-50Hz across amplitudes ranging from 0.1mA to 6mA. Throughout stimulation, myoelectric responses were recorded bilaterally using surface electromyographic (EMG) electrodes over eight major muscles on both the intact and residual limb. Compound muscle action potentials (CMAPs) were derived from the stimulation-triggered average responses generated for each stimulation parameter. The subjects reported the location and quality of any elicited sensations using a custom graphical user interface. CMAP amplitudes increased with increasing stimulation amplitudes. Muscles innervated in more rostral segments, i.e. the quadriceps, of the spinal cord showed higher activity during stimulation from rostral contacts, while muscles innervated in more caudal spinal segments, i.e. lateral gastrocnemius, showed higher activity during stimulation from caudal contacts. Across all electrodes, 3-5 synergies, aligned to anatomical groups, could be calculated from the evoked CMAPs. For higher-frequency stimulations, we noticed that stimulation from caudal contacts evoked percepts in the phantom foot. This was correlated to an increased EMG response in all muscles. We also observed a higher EMG activity whenever the reported sensation was centered on the anterior part of the sole. This correlation persisted strongly for all muscles. In order to develop a clinically relevant stimulation protocol that effectively restores foot and leg sensations, the normal activity of the leg muscles needs to be preserved within a physiological range. These preliminary results show that evoked sensory percepts are linked to lower-limb myoelectric reflex responses. Further work is needed to decompose this relationship to understand the predictive power of coordinated muscle activation on evoked sensation as well as how to selectively modulate both independently.

Disclosures: D. Sarma: None. **B. Barra:** None. **A.C. Nanivadekar:** None. **B. Petersen:** None. **R. Bose:** None. **E. Helm:** None. **M. Boninger:** None. **M. Capogrosso:** None. **L.E. Fisher:** None. **D.J. Weber:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.03

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1U18EB029251

Title: Minimally Invasive Delivery of an Injectable Electrode in a Large Animal Model of Dorsal Root Ganglia Stimulation

Authors: J. K. TREVATHAN[1], B. E. KNUDSEN[1], S. NIEUWOUDT[4], S. PERIYASAMY[2], A. N. DALRYMPLE[5], M. FRANKE[4], D. J. WEBER[6], A. J. SHOFFSTALL[7], K. A. LUDWIG[3]; [1]Biomed. Engin., [2]Sch. of Med. and Publ. Hlth., [3]Dept. of Biomed. Engin., Univ. of Wisconsin, Madison, WI; [4]Neuronoff Inc., Cleveland, OH; [5]Dept. of Mechanical Engin., Carnegie Mellon Univ., Madison, WI; [6]Mechanical Engieering, Carnegie Mellon Univ., Pittsburgh, PA; [7]Dept. of Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Implanted neural stimulation devices, known as bioelectronic medicines, electroceuticals, or neuromodulation therapies, are being used to treat a variety of disorders. One increasingly prevalent application of neural stimulation is the treatment of chronic pain through electrical stimulation of the spinal cord or dorsal root ganglion (DRG). Compared to spinal cord stimulation (SCS), DRG stimulation has shown improved efficacy but also an increased complication rate. This is potentially due to a complex insertion procedure where the lead is steered laterally from the epidural space and through the vertebral foramen, as well as the need to implant a pulse generator resulting in the complete system having multiple potential failure points.

To meet the need for a simpler and minimally invasive neuromodulation system, we have previously developed an injectable electrode system, referred to as the Injectrode, that can be delivered through a needle and conforms to the target neuroanatomy. We demonstrated that an Injectrode can have similar impedances and charge injection properties to conventional neuromodulation electrodes and can be used for neural stimulation. Here, we expand upon our previous work by demonstrating a procedure for minimally invasive delivery of the Injectrode to the DRG from a lateral approach performed under fluoroscopy guidance. We also compared thresholds for sciatic nerve activation between stimulation with the Injectrode and a conventional DRG stimulation electrode.

Experiments were performed in 40-50kg swine to assess delivery and function of the Injectrode

in a clinically-relevant animal model. Gold or platinum Injectrodes were delivered under fluoroscopic guidance through a transforaminal approach, while conventional DRG electrodes (St. Jude Medical) were inserted through the epidural anterograde approach normally used for DRG lead placement. Placement was confirmed with cone beam computed tomography (CBCT). To compare function, evoked compound action potentials (ECAPs) were recorded from cuff electrodes placed on the sciatic, tibial, and common peroneal nerves and used to compute recruitment curves for activation of Aβ fibers.
Our results demonstrate minimally invasive delivery of the Injectrode in a swine model with clinically-relevant spine anatomy. We also show similar thresholds for recruitment of Aβ fibers between stimulation applied through the Injectrode and conventional DRG electrodes. By reducing invasiveness of the neuromodulation system and complexity of the surgical procedure, the Injectrode fills an unmet need for less invasive neuromodulation electrodes that can stimulate complex neural targets.

Disclosures: **J.K. Trevathan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc.. F. Consulting Fees (e.g., advisory boards); Iota Biosciences. **B.E. Knudsen:** None. **S. Nieuwoudt:** A. Employment/Salary (full or part-time):; Neuronoff Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc.. **S. Periyasamy:** None. **A.N. Dalrymple:** None. **M. Franke:** A. Employment/Salary (full or part-time):; Neuronoff Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc. **A.J. Shoffstall:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc. **K.A. Ludwig:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc.. F. Consulting Fees (e.g., advisory boards); NeuroOne Medical Inc., Cala Health, Blackfynn, Battelle, Galvani.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.04

Topic: E.05. Brain-Machine Interface

Title: Modelling recruitment of afferents in the dorsal root ganglion for somatosensory neuroprostheses

Authors: *J. FAROOQUI[1,2], A. C. NANIVADEKAR[3], M. CAPOGROSSO[4], S. F. LEMPKA[6], L. E. FISHER[5];
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Abstract: Electrical stimulation of the dorsal root ganglion (DRG) is a promising intervention to restore sensory feedback in the missing limb for individuals with amputations. Epineural electrodes, placed on the surface of the DRG, are an attractive tool for such stimulation due to their stability and reduced likelihood of causing tissue damage, as compared to penetrating electrodes which pierce the neural tissue. Our recent work has shown that DRG stimulation delivered via epineural macroelectrodes can achieve a similar degree of selectivity compared to stimulation delivered via penetrating microelectrodes.
In this work, we developed a computational model of an L6 DRG populated with multicompartment models of pseudounipolar Aα and Aβ neurons, to probe the mechanisms of selectivity for stimulation delivered via epineural and penetrating electrodes. Our model replicated the realistic geometry of DRG neurons and realistic distributions of neuron fiber types and diameters within the DRG. We then used this model to simulate stimulation with experimentally relevant parameters.
Our results indicate that the unique geometry and spatial organization of DRG neurons may lead to different activation sites during stimulation applied by epineural and penetrating electrodes. Specifically, stimulation via epineural electrodes results in greater recruitment at the initial segment of the stem axon, adjacent to the cell body, while stimulation via penetrating electrodes preferentially recruits T-junctions. Combined with the varying density of initial segments and T-junctions near the epineural and penetrating electrodes, these recruitment differences can explain the similar selectivity observed experimentally between the epineural macroelectrodes and the penetrating microelectrodes. Further, our results suggest different recruitment properties between Aα and Aβ neurons, especially via epineural electrodes, which could suggest a possible approach for evoking different modalities of sensations.

Disclosures: J. Farooqui: None. **A.C. Nanivadekar:** None. **M. Capogrosso:** None. **S.F. Lempka:** None. **L.E. Fisher:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.05

Topic: E.05. Brain-Machine Interface

Support: NIH grant 1-U18-EB029251-01

Title: Dorsal Root Ganglion Stimulation with the Injectrode

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Abstract: Neurostimulation of the spinal cord and, more recently, the dorsal root ganglion (DRG) is being increasingly used as a neuromodulation therapy for chronic pain. These neurostimulation methods likely provide pain relief through the gate control theory of pain, where the activation of Aβ fibers inhibit the transmission of nociceptive signals in the spinal cord. DRG stimulation relieves pain in precise dermatomes, but the implant procedure is more challenging because the leads need to be steered laterally into the foramen. The implant procedure may be simplified by using a more flexible and conformal electrode. In this study, we tested the Injectrode as an alternative electrode for DRG stimulation. The Injectrode comprises a conducting polymer that is injectable, cures in place, and conforms to a neural target. We injected the Injectrode onto the L6 and L7 DRG in 4 anesthetized cats either through a burr hole in the lamina or a partial laminectomy. DRG stimulation comprised of biphasic pulses with a pulse width of 80, 150, or 300 μs using either the Injectrode or a stainless steel electrode. We recorded evoked compound action potentials (ECAPs) from the sciatic, tibial, and common peroneal nerves. We measured ECAP thresholds and recruitment rates and calculated their conduction velocity. We also compared the electrochemical impedance spectra of the Injectrode and stainless steel electrode in vivo. Here we show that DRG stimulation using the Injectrode can excite primary afferents with conduction velocities ranging from 13-120 m/s, corresponding to Aα, Aβ, and Aδ fibers. The Injectrode had similar or lower ECAP thresholds than the stainless steel electrode. The threshold for evoking ECAPs from Aβ fibers were 90.9 ± 42.9 nC for the Injectrode and 100.0 ± 32.3 nC for the stainless steel electrode ($p < 0.05$). Larger diameter fibers were activated at lower amplitudes (Aα, Aβ, then Aδ). The Injectrode had similar recruitment rates as the stainless steel electrode. At 50% of the maximum stimulation amplitude, ECAPs from Aα and Aβ were most prevalent (in 100% and 75% of trials respectively), with very few ECAPs from Aδ fibers (5.4% of trials). Impedances for both the Injectrode and stainless steel electrode were similar at higher frequencies (>1 kHz) and <1 kΩ. At low frequencies (<100 Hz), the impedance of the Injectrode was lower and more resistive than the stainless steel electrode. The Injectrode can excite primary afferents, notably Aβ fibers, when used for DRG stimulation. The Injectrode can be implanted using a simple technique while achieving similar activation to conventional electrodes, making it a worthy candidate for DRG stimulation or other neuroprosthetic applications.

Disclosures: A.N. Dalrymple: None. **J.E. Ting:** None. **R. Bose:** None. **J.K. Trevathan:** None. **S. Nieuwoudt:** A. Employment/Salary (full or part-time):; Neuronoff, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Neuronoff, Inc. **M. Franke:** A. Employment/Salary (full or part-time):; Neuronoff, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff, Inc. **K.A. Ludwig:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff, Inc, NeuroOne Medical, Inc. F. Consulting Fees (e.g., advisory boards); Cala Health, Blackfynn, Battelle, Galvani. **A.J. Shoffstall:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff, Inc. **L.E. Fisher:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff, Inc, Bionic Power, Inc.. F. Consulting Fees (e.g., advisory boards); Blackfynn, NeuroOne, Inc., Battelle, Iota Biosciences.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.06

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS Grant UH3NS100541
NIH NICHD Grant F30HD098794

Title: Effects of Lumbosacral Spinal Cord Stimulation on Balance and Gait after Trans-tibial Amputation

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Abstract: Individuals with a lower-limb amputation can experience a wide range of functional impairments and, critically, a higher risk of falls than the average population. Sensory feedback is crucial to making necessary postural corrections, and the lack of sensory feedback from the prosthesis likely contributes to these functional deficits. In this study, we use lumbosacral spinal cord stimulation (SCS) to generate sensations that are perceived to emanate from the missing limb and use those sensations to provide feedback from the prosthesis. The aim of this study is to evaluate the effects of sensory feedback on balance and gait. Two SCS leads were implanted percutaneously in the epidural space of an individual with a trans-tibial amputation, targeting the ipsilateral lumbosacral spinal cord. Psychophysical experiments were completed to determine appropriate stimulation parameters to evoke and control sensations in the missing limb. During standing and walking, plantar pressure signals for the corresponding area of the prosthesis triggered stimulation. Tests of balance (Sensory Organization Test, SOT) and gait (Functional

Gait Assessment, FGA) were performed with and without stimulation. In the SOT, participants stand on a force platform while either visual or somatosensory feedback are altered. Equilibrium scores indicate a participant's ability to stay within a normative 12° of anteroposterior (AP) sway. The FGA is a 10-item clinical measure of stability during gait. We utilized the electrode that most reliably evoked sensation in the missing limb, specifically the lateral forefoot. There were no changes in composite equilibrium scores or FGA scores with the addition of sensory feedback, however stimulation did improve performance on the most challenging SOT conditions (platform sway with altered vision or platform sway without vision) and tandem walking. With platform sway and altered vision, AP excursion and area of sway decreased with stimulation. With platform sway and no vision, AP excursion increased, however path length and equilibrium score improved. For both conditions, AP stability improved more than mediolateral stability. Notably, baseline scores for this participant were comparable to able-bodied levels and ceiling effects of these measures may be a confounding factor. The participant's balance performance varied widely across trials with stimulation, indicating the need for more training prior to assessment. In addition, these outcome measures may not be sufficiently sensitive to characterize the effects of impaired sensation in this population, and therefore, may not be able to detect changes due to the addition of sensory feedback.

Disclosures: B.A. Petersen: None. **A.C. Nanivadekar:** None. **D. Sarma:** None. **R. Bose:** None. **E. Helm:** None. **M. Boninger:** None. **P.J. Sparto:** None. **D.J. Weber:** None. **L.E. Fisher:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.07

Topic: E.05. Brain-Machine Interface

Support: NIG Grant UH3NS100541

Title: Restoring sensations by percutaneous spinal cord stimulation in people with lower limb amputation

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Abstract: Sensory feedback is crucial for tasks involving the lower limbs such as balance and gait. People with lower-limb amputation lack feedback from the missing limb, and often experience abnormal gait patterns, impaired balance, lack of reflexive muscle activity, and are more prone to falling. A major goal of our research is to restore sensory feedback via lower limb prostheses by electrically stimulating sensory nerve fibres in the spinal cord that project from the missing limb. Importantly, for this technique to be effective, the restored sensations should be stable across days and, ideally, they should be perceived as 'natural' (e.g. touch or movement) rather than paresthetic (e.g. electrical buzzing). In this context, we evaluated (1) the locations and focality of percepts evoked by stimulating the ipsilateral lumbosacral spinal cord, (2) the stability of these percepts over 4 weeks, and (3) the perceptual quality (i.e modality and intensity) of these percepts.We implanted two or three 16-contact spinal cord stimulation (SCS) leads in the epidural space of the lumbosacral spinal cord in two human subjects with transtibial amputation over a period of 29 days. We delivered stimulation through monopolar and multipolar combination of contacts over a wide range of stimulation parameters (e.g. amplitude, frequency) and recorded the location, modality, and intensity of the evoked sensations. We also conducted standard psychophysical assessments to measure the just noticeable difference and threshold for evoking a percept.In both subjects, lumbosacral SCS elicited sensations localized to the ankle and foot of the missing limb along with sensations in the residual limb. The threshold for evoking sensations in the missing limb decreased over time but was always higher than the threshold for evoking sensation in the residual limb. A majority of percepts evoked in Subject 1 were described as naturalistic while Subject 2 reported a majority of percepts as paresthetic. There was substantial lead migration with Subject 1, but changes to the surgical approach minimized migration for Subject 2, and the evoked percepts displayed greater stability over the 29-day implant period. We demonstrated that percutaneous SCS can elicit sensory percepts in the missing limb for the two people with lower-limb amputation. The stability of the sensations was also improved by the surgical procedure of anchoring the SCS leads. We plan to recruit additional subjects and perform closed-loop experiments to observe potential improvements in balance and gait over a longer timescale.

Disclosures: R. Bose: None. **A.C. Nanivadekar:** None. **B. Petersen:** None. **D. Sarma:** None. **E. Okorokova:** None. **S.J. Bensmaia:** None. **E. Helm:** None. **M. Boninger:** None. **D.J. Weber:** None. **L.E. Fisher:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.08

Topic: E.05. Brain-Machine Interface

Support: UG3NS107688

Title: Technology development towards a novel auditory nerve implant (ANI)

Authors: *M. LEBER[1], J. CREW[1], L. RIETH[2], K. HÜBNER[3], C. CROSFIELD[4], A. HEILLER[5], I. SONDH[5], L. VERDUN[5], W. M. THOMAS[6], S. MASTRANGELO[6], S. BOROOMAND[6], Y. FAN[6], S. STRAHL[3], D. SIEBER[3], K.-H. DYBALLA[7], W. NOGUEIRA VAZQUEZ[7], D. J. WARREN[6], G. M. GHOSE[5], L. A. JOHNSON[5], S. ZUNIGA[5], R. FRANKLIN[1], S. NEGI[1], A. SAMII[8], A. J. OXENHAM[5], M. ADAMS[5], T. LENARZ[7], F. SOLZBACHER[6], H. H. LIM[5];
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Abstract: Cochlear implants (CIs) use an intra-cochlear multi-electrode array to electrically activate auditory neurons and have restored hearing in hundreds of thousands of deaf individuals. However, the fundamental performance of the CI has not significantly improved for over 25 years; it is generally accepted that this lack of improvement is due to the physical limitations of the electrode-nerve interface. Current spread resulting from multiple factors limits the number of effective independent channels, which in turn limits performance in the use cases of speech-in-noise and music perception. One strategy to overcome this limitation is to directly stimulate fibers of the auditory nerve. The development of a novel auditory prosthesis, an Auditory Nerve Implant (ANI), is presented in which a penetrating electrode array is directly inserted into the auditory nerve between the cochlea and the brainstem. The ANI system uses a commercially available cochlear stimulator from MED-EL but replaces the existing macro-electrode array and lead with a penetrating micro-electrode array and helically coiled lead tailored to the dimensions of the human auditory nerve. The ANI electrode array is a modified Utah Slanted Electrode Array consisting of 15 shanks in a 3x5 grid. Linearly increasing electrode shank lengths (0.5 mm - 0.9 mm) across the array allow stimulation across the auditory nerve cross-section. The helical lead improves mechanical performance (as measured by bend and tensile testing) such that it complies with mechanical standards for cochlear electrode leads. The design of the electrode array, the lead, and the insertion tool were optimized through human cadaver studies. Two surgical approaches, translabyrinthine and retrosigmoid, were proposed and their viabilities were confirmed via intraoperative experiments. Initial acute experiments in cats and non-human primates have demonstrated successful stimulation of the auditory nerve based on recordings of electrically evoked auditory brainstem responses (eABRs). Chronic non-human primate and cat experiments are currently in process to assess the long-term safety and stability of the ANI. Insertion testing will be performed using the modified ANI insertion tool and a nerve phantom model to verify the safety and proper insertion of the modified electrode array. Finally, the data collected from preclinical safety studies of chronically implanted cats and non-human primates will be used to seek regulatory approval for a clinical trial.

Disclosures: **M. Leber:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **J. Crew:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **L. Rieth:** None. **K. Hübner:** A. Employment/Salary (full or part-time):; Med-El. **C. Crosfield:** None. **A. Heiller:** None. **I. Sondh:** None. **L. Verdun:** None. **W.M. Thomas:** None. **S. Mastrangelo:** None. **S.**

Boroomand: None. **Y. Fan:** None. **S. Strahl:** A. Employment/Salary (full or part-time):; Med-El. **D. Sieber:** A. Employment/Salary (full or part-time):; Med-El. **K. Dyballa:** None. **W. Nogueira Vazquez:** None. **D.J. Warren:** None. **G.M. Ghose:** None. **L.A. Johnson:** None. **S. Zuniga:** None. **R. Franklin:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **S. Negi:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **A. Samii:** None. **A.J. Oxenham:** None. **M. Adams:** None. **T. Lenarz:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Neurotech. **H.H. Lim:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.09

Topic: E.05. Brain-Machine Interface

Support: NIH UG3NS107688
NSF UtB DGE 1734815

Title: Simulation study of a novel auditory nerve implant: model and expected percepts

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Abstract: An intracranial auditory prothesis that targets the auditory nerve between the cochlea and brainstem (auditory nerve implant, ANI) is currently being developed across five institutions and two medical device companies (MED-EL and Blackrock Neurotech). The overall goal of the ANI is to improve hearing performance over current hearing treatment options. The ANI will be directly implanted into the auditory nerve with the aim of achieving more focused activation and greater transmission of auditory information to the brain compared to current cochlear implants. The ANI consists of a MED-EL stimulator attached to a Utah Slanted Electrode Array (USEA; developed by Blackrock Neurotech) with 15 electrodes in a 3x5 configuration. A spatial model of the auditory nerve cross-section was created to mimic the anatomical organization of the fibers, overall shape, and diameter of the auditory nerve. Two tonotopic frequency organizations were tested: an ideal 2.25 turn spiral like the tonotopy observed in the cochlea and a randomized tonotopy. These 2D models of the auditory nerve fibers were translated into 3D by extending each fiber in a third dimension. The tips of the USEA were placed within these fibers based on the expected positioning of the ANI in future patients. A computational model of the neuronal activation based on cochlear implant stimulation was employed by applying an electrical current at the electrode tips to simulate excitation profiles of the auditory nerve fibers. These excitation

profiles were converted into vocoded stimuli to test the expected percepts of the ANI in 10 normal-hearing listeners to determine the potential performance of the device. The main outcomes measured with these stimuli were threshold, dynamic range, loudness balancing, and pitch ordering. The excitation profiles showed a variety of shapes ranging from single frequency peak with a narrow spread of activation to multiple peak frequencies with broader spread of activation. Threshold and loudness balancing levels were determined for each of the simulated electrodes for each of the listeners. The loudness-balanced stimuli were used in the pitch-ordering task under two conditions: no level roving and 2 dB level roving. The results of the pitch-ordering tasks indicated that it was possible to map the simulated electrodes to the appropriate frequency bands, even with the multiple frequency peaks observed on some electrodes. The results of these studies will aid in the future development of perceptual tasks for the ANI clinical trial.

Disclosures: A.P. Heiller: None. **K. Dyballa:** None. **D. Kipping:** None. **B. Richardson:** None. **T. Lenarz:** None. **A.J. Oxenham:** None. **W. Nogueira:** None. **H.H. Lim:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.10

Topic: E.05. Brain-Machine Interface

Support: NIH UG3NS107688
NSF UtB DGE 1734815

Title: Characterization of electrically-evoked auditory brainstem responses in rhesus monkey, cat, and guinea pig produced by stimulation via a novel auditory nerve implant using the Blackrock Utah array

Authors: *I. SONDH[1], G. M. GHOSE[1], L. A. JOHNSON[1], L. VERDUN[1], D. J. WARREN[2], W. M. THOMAS[2], L. RIETH[3], M. LEBER[4], F. SOLZBACHER[2,4], S. ZUNIGA[1], M. ADAMS[1], H. H. LIM[1];
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Abstract: The cochlear implant (CI) is an effective neural prosthetic capable of restoring hearing in those with severe to profound hearing loss. However, CI users have reduced performance in noisy environments and music perception. A major cause for these deficiencies is the poor electrode-neural interface due to the bony cochlear wall, which causes unwanted current spread across the frequency coding of the cochlea. Recent studies have shown that direct stimulation of the auditory nerve could help overcome these limitations and activate a greater number of

frequency channels of information. Therefore, our group is developing and testing a new auditory nerve implant (ANI) in several animal models. To demonstrate successful activation of the auditory system using the ANI, acute experiments were performed in the cat (n=4) and rhesus monkey (n=2) using a 3x5 Utah Slanted Electrode Array (USEA) developed by Blackrock Neurotech. The USEA was implanted in the auditory nerve via a translabyrinthine surgical approach. Needle electrodes placed in the scalp and mastoid areas were used to record the electrically-evoked auditory brainstem response (eABR). In both species, multiple component waves of the eABR were observable, indicating strong activation of auditory brainstem structures. Using different stimulation sites on the array resulted in changes in the eABR waveform, suggesting activation of different nerve fiber groups per site. The amplitude of the eABR waves increased with applied current level and the latency of eABR peaks decreased, showing progressively stronger recruitment of nerve fibers. Guinea pig studies were also performed to support the findings in our cat and monkey studies. Because the smaller skull of the guinea pig prevented usage of the USEA, we utilized a 16-site single-shank probe developed by NeuroNexus. In addition to the eABR, invasive recordings of multi-unit activity were collected, through a 32 site 2-shank probe placed along the tonotopic axis of the central nucleus of the inferior colliculus (ICC). The ICC recordings in response to stimulation corroborated the eABR recordings; only certain groups of ICC neurons (i.e., only those tuned to certain frequency ranges) were activated, depending upon stimulation site. This supports the assertion in the cat and monkey studies that changes in the eABR waveform were due to recruitment of different frequency fiber groups. Future experiments will involve chronic implantation in 2 NHPs, with eABRs being measured periodically to track stability, functionality, and safety of the device. We then seek to translate to the clinical setting and ultimately provide a solution for patients who are not eligible for a CI.

Disclosures: I. Sondh: None. **G.M. Ghose:** None. **L.A. Johnson:** None. **L. Verdun:** None. **D.J. Warren:** None. **W.M. Thomas:** None. **L. Rieth:** None. **M. Leber:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Neurotech. **S. Zuniga:** None. **M. Adams:** None. **H.H. Lim:** None.

Poster

P557. Neuroprosthetics: Other Motor Sensory Interfaces: E.G. Artificial Vision I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P557.11

Topic: D.07. Vision

Support: NIH Grant 1R01EY029741-01

Title: Dynamic Epiretinal Stimulation Improves Shape Discrimination in People with Blindness

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Abstract: Different neural targets along the visual pathway can be stimulated to elicit flashes of lights known as phosphenes that can be used as feedback for people with blindness. The information delivered by a single phosphene is limited, so phosphene presentations are often spatially combined through electrode array stimulation to maximize real-time visual understanding of one's environment. In theory, stimulating electrodes that form a specific shape within an array should elicit phosphenes in the same shape, given that the retina and primary visual cortex (V1) are retinotopic. However, it is not typically that simple: when multiple electrodes are stimulated simultaneously, phosphenes tend to merge together in an uninterpretable way (Bosking et al. 2018). To address this, a recent study demonstrated that shapes could be "drawn" by sequentially stimulating electrodes in V1 (Beauchamp et al. 2020). To evaluate if this technique would be successful when the retina is the neural target, we conducted a similar study in people with epiretinal microelectrode arrays.
We conducted experiments with two subjects with retinitis pigmentosa. These individuals had previously been implanted with the Argus II retinal prosthesis, which consists of a 6x10 electrode array (Luo et al. 2016). We ran a two-alternative forced choice (2-AFC) discrimination task in which subjects were asked to respond if an arrow-shaped simulation on the array was pointing to the left or to the right. These arrow-shapes were created by stimulating three electrodes that evoked phosphenes which were perceived as similar in size, shape, and brightness by the subjects. We ran this test with either "static" stimulation (all electrodes were stimulated simultaneously for 200 ms) or "dynamic" stimulation (electrodes were sequentially stimulated, each for 200 ms with no temporal gap in between).
Our results indicate that dynamic stimulation outperforms static stimulation when discriminating arrow direction. When performing the test with dynamic stimulation, both subjects got 19/20 trials correct. When performing the task with static stimulation, one subject achieved only 4/20 correct and the other 8/20 correct. Interestingly, one subject originally found it impossible to make a judgment until we used electrodes that elicited similar phosphene shapes and sizes.
Our next steps are to recruit additional subjects and to test dynamic stimulation with more complex shapes, such as letters of the alphabet. Our preliminary findings demonstrate that an epiretinal prosthesis can produce coherent simple shapes with a dynamic stimulation paradigm, demonstrating their functionality to restore rudimentary vision.

Disclosures: B. Christie: None. **R. Sadeghi:** None. **A. Kartha:** None. **S. Lee:** None. **A. Caspi:** None. **F. Tenore:** None. **R. Klatzky:** None. **G. Dagnelie:** None. **S. Billings:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.01

Topic: E.06. Posture and Gait

Support: A Cure for Ellie Foundation
NIH U54NS115052
NIH US4HD079123
NIH T32 HD007414

Title: Wearable Sensors Detect Impaired Balance and Coordination in LBSL During Remote, Home-Based Assessments

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Abstract: Objective The objective is to identify neuromotor measures that can detect disease severity in the disease leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL). LBSL is a rare neurologic disorder with motor impairment due to biallelic mutations in *DARS2,* which encodes mitochondrial aspartyl tRNA synthetase. There are white matter changes in the cerebrum, cerebellum, pyramidal tracts and dorsal columns, resulting in ataxia and spasticity. There are no targeted therapies for LBSL. Methods The study analyzes cross-sectional data from wearable sensors and clinical scales used to assess walking and balance in LBSL and age-matched control participants' homes through video conferencing. Outcomes include parameters obtained from a wearable accelerometer system for gait and static postural sway in 4 standing conditions and the Scale for the Assessment and Rating of Ataxia (SARA). Measures were collected on two consecutive days in a subset of participants for the reliability analysis. Results A total of 8 LBSL participants and 8 controls were included in the study, age range 5 - 23 years. There is good reliability of remotely collected gait measures (intra-class correlation coefficient 0.86-0.99) and sway measures (intra-class correlation coefficient 0.53-0.96) among participants. LBSL participants have a slower stride velocity (1.11±.03 vs. 1.02±.03m/s) and shorter stride length (1.11±.02 vs. 1.05±.02m) than their control counterparts. Lateral step variability, which indicates variability in walking step width, is increased for LBSL participants (5.6±0.6 vs. 3.7±0.4cm). During standing conditions with the eyes closed and feet together, LBSL participants had anteroposterior sway instability covering a much greater area than controls (2.1±0.6 vs. 0.3±0.1 m^2/s^4). These variables correlate strongly with the SARA (r=0.6-0.9). Conclusions Virtual collection of data in the home using wearable sensors is a reliable and sensitive means for detecting gait and balance abnormalities LBSL patients. Distinct gait and balance abnormalities measured in the LBSL patients are related to clinical ratings of ataxia severity. This work will address the unmet need to establish quantitative outcome measures in the natural history of LBSL.

Disclosures: **A. Smith Fine:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **J. Goodman:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **B.R. Turk:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **A.J. Bastian:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH T32 HD007414 NIH R01 HD040289 NIH R37 NS090610 Joey's Foundation. **A. Fatemi:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH US4HD079123, NIH U54NS115052, A Cure for Ellie Foundation. F. Consulting Fees (e.g., advisory boards); Paid member of drug safety monitoring board – Bluebird Bio. **J. Keller:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. Other; Independent Contractor, New Beginning Pediatric Rehabilitation.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.02

Topic: E.06. Posture and Gait

Title: Wearable Inertial Measurement Units Demonstrate Strategy and Performance Differences During Individual Ice Dance Steps

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Abstract: Background:The ability to continuously identify and quantitatively rate motor activity in patients with movement disorders could provide a more granular view of disease activity for clinical management or clinical trials. Our objective was to demonstrate the feasibility of collecting and classifying movement data efficiently from stereotyped ice dance patterns and to demonstrate that meaningful conclusions could be drawn from the data. Ultimately, this approach could be used to develop a training set for a machine-learning algorithm to detect, classify, and analyze movements from a continuous data stream. Methods:Three ice skaters each performed three patterns of the Dutch Waltz, a basic ice dance pattern. Noitom Perception Neuron wearable inertial measurement units (IMUs) were used to collect movement data, and video recordings were used to verify accurate execution of steps. Traces were visualized according to step classification, and were compared to average traces using Pearson's product-moment correlation coefficient. Classification utility was illustrated using a receiver operator

characteristic curve. Skating quality was visualized using Fourier analysis. Results:The ice dance pattern data from a limited subset of sensors clearly showed it was possible to visually differentiate general features in an ice dance pattern as well as skater-specific differences. Correlation analysis showed that individual movements were more likely to be correlated with a template step of the same classification vs a different classification. Receiver operator characteristic analysis of classification utility using a personalized template demonstrated an area under the curve up to 0.915. Moreover, hip instability observed on video could be detected in the waveform of the traces and on Fourier analysis as high-frequency hip oscillations. Conclusion: Our results show it is feasible to collect data from wearable motion sensors during ice dance pattern dances. Moreover, the data are grossly reproducible; they correspond to identifiable physical features in the dance and identify performance features that are meaningful for coaching and also suggest a different strategy for performance. Although classification of steps in a single individual could be robust, generalized classification may be optimized either by using an individualized template or by using different features. It will be essential to examine a wider range of individuals to understand the spectrum of individual variation and learning trajectories, as well as to gather a complete training set for an automated movement recognition algorithm to recognize a greater variety of movements.

Disclosures: S.G. Ying: None. **Y.S. Tarui:** None. **A.V. Joyce:** None. **A.G. Ying:** None. **C. Dugan:** None. **A.T. Joyce:** None. **V.A. Ye:** None. **M.E. Dugan:** None. **N.S. Ying:** None. **C.F. Joyce:** None. **D. Dugan:** None. **S. Pan:** None. **M. Bramante:** None. **E. Geminiani:** None. **S.H. Ying:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.03

Topic: E.06. Posture and Gait

Support: REACT Center of NIH/NICHD [RFA-HD-15-010]
Brooks-PHHP Research Collaboration
NIH National Center for Medical Rehabilitation Research T32 Neuromuscular Plasticity Training Program [T32HDO43730]
NIH/NICHD K12 Rehabilitation Research Career Development Program [HDO55929]

Title: Effects of transcutaneous spinal direct current stimulation applied during walking rehabilitation on spinal reflex modulation, reflex behavior and walking function

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Abstract: Spinal cord injury (SCI) alters the excitability of spinal circuits below the injury, affecting motor output and reflex modulation in a manner that contributes to walking dysfunction. Studies of neuromodulatory electrical stimulation approaches demonstrate beneficial effects on spinal motor output; however, these approaches can be painful, have unwanted side effects or require invasive procedures. In contrast, transcutaneous spinal direct current stimulation (tsDCS) uses a non-invasive, low-dose current applied with an affordable, commercially available device. Importantly, tsDCS can modulate spinal reflexes, increase corticospinal excitability, and influence spinal processing of sensory inputs. Therefore, tsDCS may enhance the responsiveness of these circuits to synaptic inputs associated with task-specific rehabilitation. We tested the hypothesis that a single session of tsDCS applied during walking rehabilitation (tsDCS+walk), compared to sham stimulation during rehabilitation (sham+walk), induces improvement in spinal modulation. We also examined changes in reflex behavior and walking function after 16 sessions. Eight individuals with chronic motor incomplete SCI (6 male, 31-62 years, speed 0.07-0.83 m/s, 6 wheelchair dependent) completed two single-day, random-ordered sessions of either tsDCS+walk (anodal tsDCS, 2.5 mA, 30 min) or sham+walk. Spinal modulation was assessed through post-activation depression (PAD) of the soleus Hoffman (H) reflex, elicited supine with a 1 ms pulse to the tibial nerve. PAD was calculated as the percent reduction in amplitude of a second H reflex stimulated 1 second after the first. Four participants had recordable reflexes. PAD increased an average of 10.5±7.2% with tsDCS+walk with all 4 demonstrating improvement compared to 1 of 4 improving after sham+walk (average change -6.2±8.5%). Participants were randomized to receive 16 sessions of tsDCS+walk or sham+walk. Session goals were 30 minutes of treadmill and 10 minutes of overground walking at high intensity. Pre- and post-training, we completed a double-blind assessment of reflex behavior (clonus subscale of the Spinal Cord Assessment Tool for Spastic Reflexes) and walking function (walking speed). Clonus improved in 2 of 4 receiving tsDCS+walk compared to 1 of 4 in sham group. Speed gains exceeded the minimal clinically important difference (>0.13 m/s) in 3 of 4 receiving tsDCS+walk and 1 of 4 in the sham group. In a small cohort, greater gains were evident with tsDCS+walk versus sham+walk. Outcomes suggest this non-invasive stimulation may have beneficial effects on spinal modulation and clinical outcomes, warranting further investigation.

Disclosures: K.A. Hawkins: None. **D.D. Fuller:** None. **D.J. Clark:** None. **E.J. Fox:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.04

Topic: E.06. Posture and Gait

Support: NIH R21 NS114816
PF-JFA-2036

Title: Vector coding technique to quantify arm swing coordination during walking in individuals with Parkinson's disease

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Abstract: Although reduced arm swing amplitude is a common motor feature of Parkinson's disease (PD) it is unclear if swing coordination between arms is also impaired during gait. A better characterization of arm swing coordination is crucial because coordination between body segments during gait can be an indicator of a healthy functioning system. Limb coordination is commonly assessed in the time domain, however, vector coding of interlimb segments in angle-angle space can describe different relationships not observed in the time domain. Therefore, the aim of this study is to evaluate arm swing coordination in individuals with PD and healthy older adults during gait using vector coding and circular statistics. Twenty-one healthy older adults (11 female, 63.28 +/- 9.86 yrs) and eleven individuals with PD (3 female, 59.72 +/- 9.38 yrs) participated in the study. Individuals with PD were tested off antiparkinsonian medication (>12h withdrawal). Participants performed a 2-minute walking task up and down a corridor (7.6m) for three conditions: preferred walking speed, preferred walking speed with a cognitive task of counting backwards by threes, and a faster walking speed. Participants wore an APDM Opal inertial measurement unit set consisting of a sensor strapped to the participant's lumbar spine, sternum, left and right wrist, and left and right foot. Angular velocities around the medial-lateral axis were extracted from both wrists and used to calculate the coupling angle at each point of the gait cycle in the vector coding approach. Circular variance of the coupling angle was calculated at each point of the gait cycle, then averaged across the gait cycle. As a secondary analysis the correlation between the angular velocity time series at each wrist was calculated. For both outcome variables a two-way ANOVA was performed to analyze effects of group and condition. Results show that individuals with PD have a higher circular variance of the coupling angle and lower correlation coefficients between arms than healthy older adults during the cognitive task condition further suggesting that individuals with PD lack arm swing coordination during gait with a dual task. Therefore, arm swing coordination may serve as an additional assessment of PD and a target for mobility goals that can be addressed in a clinical setting.

Disclosures: M. Arcodia: None. **J. Chung:** None. **J.J. Jeka:** None. **H. Reimann:** None. **R.G. Burciu:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.05

Topic: E.06. Posture and Gait

Support: NIH Grant EB024570
NIH Grant R01HD098154

Title: Determination of chief features in clinician observational gait analysis

Authors: *S. SETHI, J. SCHIERLMANN, I.-C. LEE, H. HUANG;
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Abstract: Observational gait analysis (OGA) is an important skill for clinicians when evaluating gait abnormality. However, various factors such as patient height or fitness may inadvertently affect clinician judgment. Consequently, little is known about which objective gait features they rely on during OGA. To answer these questions, we propose a novel method to (1) identify what body areas clinicians gaze at to diagnose gait abnormality, (2) investigate the difference in OGA skill between individuals with different levels of clinical experience, and (3) determine what factors affect their accuracy.

To answer these questions, the motion of three unilateral above-knee amputees and a healthy control walker were utilized to build three biological motion models through principal component analysis. For each amputee model, we meshed the gait features between the amputee and control walker to build 6 videos that displayed synthesized point-light walking motion. We varied the weight of gait features from 0 to 100% in 20% increments, beginning with amputee gait at 0% and ending with healthy gait at 100%. These videos were used to test the accuracy in judging the range of gait abnormality for two groups of participants—experienced raters with at least 2 years of clinical experience, and inexperienced raters. Gaze was recorded with an eye tracker system as participants watched 10 trials of video sets generated from each amputee.

All participants fixated on the left side of the pelvis most overall ($p < 0.05$), and the more experienced participants fixated on the left shoulder more than the inexperienced participants ($p < 0.05$). No other factors were found to influence gaze. Regarding accuracy, Amputee A (K-3 level) was the easiest for all participants to rank ($p < 0.05$); Amputee B, which was the same K-level as Amputee C (K-4) but possessed much less joint asymmetry, was significantly more difficult to rank ($p < 0.05$). More experienced participants were more accurate in rating Amputee B than inexperienced participants ($p < 0.05$). However, we did not find a difference in accuracy based on experience when rating the other amputees. Moreover, participants consistently ranked walkers with very abnormal gait (0% and 20% control walker motion) more accurately than walkers very close to normal motion (60%, 80%, and 100% control walker motion; $p < 0.01$). These results indicate that hip motion is the most common feature that was used by both groups in judging gait abnormality. Furthermore, the clinicians may gain greater accuracy by evaluating shoulder motion. Overall, the degree of gait abnormality is the largest factor affecting accuracy.

Disclosures: S. Sethi: None. **J. Schierlmann:** None. **I. Lee:** None. **H. Huang:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.06

Topic: E.06. Posture and Gait

Support: This project has been funded by the Jump ARCHES endowment through the Health Care Engineering Systems Center.

Title: Effect of Aging on Cortical Activity in Response to Sensory Organization Test

Authors: *Y. HU[1], A. BISHNOI[1], L. ZIEGELMAN[2], M. E. HERNANDEZ[1];
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Abstract: Decreased upright postural control ability is associated with aging, leading to a higher risk of falling, decreased mobility, decreased functional capacity and independence, and reduced ability to perform physical activities, thus reducing older adults' quality of life. Although previous evidence suggests cortical involvement in human postural control, it is unclear how aging affects functional cortical activity. The current study examined cortical electrical activity in response to sensory perturbations that challenge standing postural control. Cortical beta rhythm (13-30hz) activity at postural control-related areas (electrodes Fz, Cz, Pz) were measured by Electroencephalography (EEG) in older adults (OA) and young adults (YA). We hypothesized that 1) OA would demonstrate higher beta activities compare to YA; 2) there would be a significant age by condition interaction, in which YA would demonstrate increased beta activity with increased difficulty in a condition, while OA would not demonstrate any changes. In a cross-sectional study design, we examined 13 OA (71±6yrs; Sex: 7F/6M; BMI:25) and 10 YA (22±2yrs; Sex: 4F/6M; BMI:25). Participants completed baseline cognitive and motor testing before starting the clinical sensory organization test (SOT) on a Neurocom Clinical Research System. In SOT, participants completed 3 trials each of 6 conditions: Eyes open, Eyes closed, Eyes open sway-referenced surround, Eyes open sway-referenced platform, Eyes closed sway-referenced platform, Eyes open sway-referenced surround and platform. The participants were instructed to maintain upright balance without taking steps throughout each condition. The results of linear mixed effect models analysis revealed age ($p<0.05$), condition ($p<0.01$), and electrode effects ($p<0.05$), and age by condition interaction ($p<0.01$). In addition, post hoc analysis confirmed the interactions between age and condition. We found statistically significant higher beta power in OA compares to YA. Moreover, while YA increased beta power as condition difficulty increased, OA demonstrated the opposite behavior. Larger beta depression associated with eye-close conditions was also found in OA in comparison to YA. This study suggests aging altered cortical engagement in postural control of standing balance tasks,

indicating potential cortical hyperactivity and higher voluntary control in response to sensory balance perturbations in OA.

Disclosures: Y. Hu: None. **A. Bishnoi:** None. **L. Ziegelman:** None. **M.E. Hernandez:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.07

Topic: E.06. Posture and Gait

Support: R21NS119849
R01 NS058487
R21 NS096258

Title: Reducing the midline tremor with deep brain stimulation improves gait and balance in essential tremor patients

Authors: *Y. CHOI[1], B. YACOUBI[1], A. CASAMENTO-MORAN[1], S. DELMAS[1], B. WILKES[1], C. W. HESS[2], A. SHUKLA[2], M. OKUN[2], K. FOOTE[2], D. VAILLANCOURT[1,2,3], E. CHRISTOU[1,2];
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Abstract: Although more than 40% of Essential Tremor (ET) patients exhibit balance and gait impairments, the relation to their tremor remains unclear. Here, we compared tremor at various body locations during balance and gait tasks between healthy controls (HC) and ET patients undergoing deep brain stimulation treatment (ET DBS) and examined how neurostimulation effects tremor and performance in these tasks. Seventeen ET DBS and 17 age-and sex-matched HC performed a postural balance task (quiet standing task with eyes open) and an overground walking task at their preferred speed. We quantified tremor (4-8 Hz acceleration power) during the gait and balance tasks using wearable sensors (APDM) located at the wrists (upper limb), ankles (lower limb), chest (upper trunk), and lumbar area (lower trunk). We focused on sway path length and stride length variability for postural balance and gait performance, two variables that relate to the fall risk. ET DBS exhibited significantly greater sway path length and stride length variability relative to HC ($p<0.003$). Their tremor was greater at the lower limb and lower trunk during the postural balance task ($p<0.05$), and at the upper limb and lower trunk during the gait task relative to HC ($p<0.03$). During the postural balance task, neurostimulation significantly reduced sway path length ($p=0.01$) and lower trunk tremor ($p=0.03$). During the gait task, neurostimulation significantly reduced stride length variability ($p=0.04$). The reduction in sway path length significantly associated with a reduction in upper trunk tremor ($R^2=0.24$, $p=0.03$),

whereas the reduction in stride length variability strongly associated (R^2=0.79, p=0.001) with a reduction in both upper trunk (part r = 0.4) and upper limb tremor (part r = 0.3). Our study provides novel evidence that DBS may effectively improve gait and balance performance in ET patients by reducing trunk (midline) tremor. Trunk tremor may be an indicator of midline tremor, often associated with cerebellar degeneration, and its reduction may improve trunk control an essential component for balance and gait performance.

Disclosures: Y. Choi: None. **B. Yacoubi:** None. **A. Casamento-Moran:** None. **S. Delmas:** None. **B. Wilkes:** None. **C.W. Hess:** None. **A. Shukla:** None. **M. Okun:** None. **K. Foote:** None. **D. Vaillancourt:** None. **E. Christou:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.08

Topic: E.06. Posture and Gait

Support: NIH T32AG062728
NIH U01AG061389

Title: Associations between neural correlates of imagined walking and community mobility: preliminary results from the Mind in Motion study

Authors: *V. SHAH[1], T. FETTROW[1], C. HASS[1], D. FERRIS[1], P. RUETER-LORENZ[2], D. CLARK[1], T. MANINI[1], R. SEIDLER[1];
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Abstract: Studies have shown associations between functional brain activity and lab-based gait measurements, where increased brain activity is associated with better gait performance. Traditionally, gait performance in laboratory settings has been quantified via gait speed, step length, and step duration. However, direct associations between functional brain activity and community mobility have been rarely examined. Community mobility is complex as it is done in an uncontrolled environment with significant temporal variability. Data from the Mind in Motion study were used to characterize brain activity and associations with community mobility data. Actigraphy and MRI data were collected from 6 younger adult (20-33 yrs old) and 7 older adult (71-86 yrs old) volunteers. We used functional magnetic resonance imaging (fMRI) to record brain activity during walking motor imagery (WMI) on increasingly difficult uneven terrain. fMRI during a N-back (NB) spatial working memory task was also collected. The NB task increased in difficulty when the number of spatial targets to recall increased. Community mobility data was collected via a hip-worn ActiGraph accelerometer, worn over approximately a 2-week period. Mean cadence measures were calculated for valid wear-days (>5 hrs/day of wear

time). A voxel-wise analysis [p(uncorrected) < 0.005, k= 10] was used to identify the correlations between functionally active brain regions during the highest difficulty of the WMI/NB tasks and mean cadence of community walking. Voxel-wise results of WMI revealed a negative correlation in the right inferior temporal lobe, and positive correlations in left inferior temporal lobe, and right superior and left middle frontal gyri. For NB, we found a negative correlation in the left inferior temporal lobe. We are continuing data collection to further investigate brain-behavior correlations of mobility and will perform group-wise correlations that will highlight potential connection between brain aging and community mobility. We also plan to determine correlations of brain structure and connectivity to community mobility. Overall, our preliminary results suggest that lower brain activity in the frontal lobe during walking could result in lower cadence in community mobility, but more data are needed to confirm this relationship. Supported by NIH U01AG061389 and NIH T32AG062728.

Disclosures: V. Shah: None. **T. Fettrow:** None. **C. Hass:** None. **D. Ferris:** None. **P. Rueter-Lorenz:** None. **D. Clark:** None. **T. Manini:** None. **R. Seidler:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.09

Topic: E.06. Posture and Gait

Support: Roybal ADRD supplement pilot grant- 2P30AG022849-11

Title: Association of balance recovery mechanisms with brain structural integrity in older adults with mild cognitive impairment

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Abstract: Background: Older adults with mild cognitive impairment (OAwMCI) exhibit subtle balance control and gait deficits which are predominantly associated with brain pathologies such as impaired white matter integrity (WMI) and reduced gray matter volume (GMV). However, the relationship between balance recovery mechanisms and neural substrates in OAwMCI remains unknown. This study thus aimed to explore the associations of volitional (self-initiated) and reactive balance (in response to an external perturbation) control with WMI and GMV. **Methods:** Twelve OAwMCI (MoCA 18-25/30, > 55 years) were examined on the limits of stability test (LOS, volitional balance via Equitest), stance perturbation test (SPT, reactive balance via ActiveStep treadmill), and underwent MRI. Forward movement (frequently performed functional activity of daily living) - LOS was quantified by maximum excursion

(maximum ability to shift one's center of gravity toward the theoretical limit [MXE-%]) and directional control (amount of movement exhibited towards the target to the movement away from the target [DCL-%]). Slip-like (prevalent type of accidental falls) perturbations -SPT was quantified by postural stability (shortest distance of the COM motion state, i.e., its position and velocity, to the theoretical boundary). WMI was quantified by fractional anisotropy (FA, movement of water molecules directionality) and GMV measured in mm^3. **Results:** For LOS, reduced forward MXE was significantly ($p<0.05$) associated with lower FA in left ($R^2=0.48$) and right ($R^2=0.71$) corticospinal tract, left ($R^2=0.46$) and right ($R^2=0.47$) corticothalamic tract, left ($R^2=0.60$) and right ($R^2=0.68$) frontopontine tract, left ($R^2=0.68$) and right ($R^2=0.77$) cingulum, anterior commissure ($R^2=0.67$), and corpus callosum ($R^2=0.65$). Reduced forward DCL was significantly ($p<0.05$) associated with reduced GMV in the left cerebellum ($R^2=0.45$), brainstem ($R^2=0.52$), left ($R^2=0.39$), and right ($R^2=0.46$) thalamus. For SPT, reduced postural stability ($p<0.05$) was significantly associated with reduced FA in the right corticospinal tract ($R^2=0.61$) and right frontopontine tract ($R^2=0.50$). Reduced postural stability was significantly ($p<0.05$) associated with reduced GMV in the brainstem ($R^2=0.69$) and right cerebellum ($R^2=0.66$). **Conclusion:** Our results indicate that structural brain integrity influences stability control in OAwMCI for both volitional and reactive balance tasks, which may share some common cortico-subcortical motor pathways and relay centers. Results also show that the integrity of descending pathways from cortical attentional centers could influence stability control for both tasks.

Disclosures: L.N. Kannan: None. **T.S. Bhatt:** None. **O.A. Ajilore:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.10

Topic: E.06. Posture and Gait

Title: Use of Erasmus Ladder to assess performance after pharmacological or neurological challenge

Authors: *D. MURPHY, P. LEACH, M. ARNOLD, A. THOMAS, A. NELSON;
Biogen, Cambridge, MA

Abstract: Neurodegenerative diseases and acute injuries to the brain can impair motor and cognitive performance. Relatively high-throughput, quantitative assessment of motor performance in rodents (i.e., RotaRod or open field activity) can capture gross motor dysfunction, but fine-tuned assessment (i.e., gait analysis or horizontal ladder performance) is time consuming and often scored subjectively. We have been working with the Erasmus ladder, a semi-automated behavioral apparatus to assess motor performance and associative motor learning in mice. The Erasmus ladder is able to capture left and right stepping patterns of mice as

they cross a horizontal ladder. To understand how the Erasmus Ladder could yield sensitive and quantitative measures of motor performance we assessed performance of mice on the Erasmus Ladder following surgical or pharmacological manipulations that have been shown disrupt coordinated movement. Stepping responses and the lateral distribution of step types were captured at each time point. Experiment 1 examined the effect of ethanol (0 or 2.5g/kg, IP) 30 minutes prior to ladder assessment in C57BL/6J male mice. Ethanol-induced performance consisted of a global increase in errors compared to performance after vehicle administration. Experiment 2, in contrast, examined performance of C57BL/6J male mice after induction of a severe unilateral cortical infarct (DH-MCAO). In our experiment, a unilateral stroke selectively impaired performance on the contralateral side of the body relative to the damaged brain regions, leaving performance on the ipsilateral side indistinguishable from sham-stroke performance. These studies are consistent with previously published studies that suggest that the Erasmus Ladder is a useful tool to measure of motor performance in mice that is sensitive widespread motor impairments but is also able to differentiate unilateral impairments, such as those caused by unilateral insult such as stroke or traumatic brain injury.

Disclosures: D. Murphy: None. **P. Leach:** None. **M. Arnold:** None. **A. Thomas:** None. **A. Nelson:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.11

Topic: E.06. Posture and Gait

Title: Differences in postural control strategies after ankle and knee injuries, measured by concurrent motor evoked potential responses in leg muscles from the motor cortex during single limb stand on firm and foam surfaces

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Abstract: About 52-60% of individuals with anterior cruciate ligament (ACL) injuries reported a history of ankle injuries. Biomechanical and neurophysiological changes have been reported after both injuries, indicating impaired sensorimotor control in lower extremities (LE). Some have speculated a potential link between chronic ankle instability (CAI) and ACL injuries; however, this has not been studied in multimodal approaches. We recruited three individuals: no injury (C01, F, 27yrs), ankle sprains (A01, F, 24yrs), and ACL injuries (K01, F, 25yrs). We asked them to perform single limb stand (SLS) on a firm and foam surface and measured 1) a degree of sways, 2) muscle activity, and 3) corticospinal excitability from the motor cortex. Muscle activity was recorded from tibialis anterior (TA), peroneus longus (PL), vastus lateralis

(VL), and semitendinosus (ST) using surface EMG. Motor evoked potentials (MEPs) of TA and non-targeted muscles (PL, VL, ST) were measured using transcranial magnetic stimulation during SLS tasks. Results were the comparisons of C01's dominant leg to A01 and K01's affected leg. K01 showed the lowest sway scores for both firm and foam surfaces. The EMG data showed that C01 dominantly activated TA (mean_28.8uV) and PL (15.2uV), A01 mainly activated PL (8.69uV), K01 dominantly activated PL (25.85uV) and TA (14.4uV) during SLS_foam. The average peak-to-peak MEPs of TA was the greatest in K01 (1598.2±344.5uV), followed by A01 (955.5±307.5uV) and C01 (192.8±80.8uV) during SLS_foam. When non-targeted muscle MEPs were normalized by TA MEP trial-by-trial, C01 showed 4 times amplified MEPs in VL on average, while A01 had similar degrees of corticospinal excitability in all muscles. K01 showed 1.3 times amplified MEPs in PL only while corticospinal excitability in VL and ST were mostly turned off. Each individual's different control strategies are not explicitly demonstrated by task performance and/or degrees of muscle activation. Measuring concurrent MEPs of non-targeted muscles may provide information on corticospinal recruitment patterns as cortical motor maps of those muscles overlap. C01 VL's amplified MEP may reflect flexibility and its ability to control instability as dynamic control increases corticospinal excitability. With CAI, the neuromuscular system may explore ways to stabilize the LE, recruiting all muscles synergically while PL plays a role in planting the ankle on the surface. Finally, an individual with both CAI and ACL injuries co-contracts TA and PL, stiffening the leg and avoids synergic use of VL and ST, which lacks flexibility to instability. These approaches may help explore a potential link between CAI and ACL injuries.

Disclosures: N. Ko: None. **C. Sano:** None. **H. Clementi:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.12

Topic: E.06. Posture and Gait

Support: New Jersey Commission on Brain Injury Research CBIR19IRG033

Title: Anticipatory and compensatory postural adjustments during perturbed standing in individuals with traumatic brain injury

Authors: *A. VEERUBHOTLA, N. EHRENBERG, O. IBIRONKE, R. PILKAR;
Kessler Fndn., West Orange, NJ

Abstract: Anticipatory postural adjustments (APA) and compensatory postural adjustments (CPA) are two types of postural mechanisms employed to achieve balance and prevent falls during perturbations. APA are the neuromechanical responses generated prior to the predictable

perturbations and their goal is to minimize the impact of the imminent perturbations. CPAs, on the other hand, are the postural responses generated after the onset of a perturbation to stabilize the body and achieve equilibrium. Failure to employ these defense mechanisms in response to perturbations can lead to postural instability and a potential fall. Traumatic brain injury (TBI) diminishes the ability to sense the environmental perturbations and impairs the ability to generate essential responses such as APA and CPA. Weakened ability to generate APA may need much larger CPA responses during perturbation to maintain stability. This could be an unattainable demand due to the neuromuscular pathology after a TBI. The generation of APA and CPA responses to perturbations is unclear in TBI populations. The objective of the current investigation is to understand APA and CPA generation post TBI and evaluate the effectiveness of a novel computerized feedback-based balance intervention to improve APA-CPA generation and functional balance. In this study, we examined APA and CPA responses during predictable and unpredictable perturbations to the base of support in healthy control (n=5) and TBI (n=10,) on a programmable platform during upright standing. Additionally, TBI-Intervention (TBI-I, n=3) group performed 16-sessions (4 days/week for 4 weeks) of balance intervention with visual and auditory feedback. The APA/CPA responses to predictable and unpredictable perturbations were derived using tibialis anterior electromyography outcomes (amplitude, reaction time) from 300ms before and after the perturbation onsets, respectively. The functional balance outcomes included Berg balance scale (BBS), and timed-up and go (TUG). The design and outcomes of the proposed study present a novel way of understanding critical factors behind balance dysfunction and falls after a TBI. Understanding APA and CPA generations during base of support perturbations is extremely vital as many of the environmental perturbations during everyday activities involve modifications to the base of support conditions. This study provides valuable knowledge based on APA and CPA generation which is a key to designing strategies to improve balance dysfunction and prevent falls post TBI.

Disclosures: A. Veerubhotla: None. **N. Ehrenberg:** None. **O. Ibironke:** None. **R. Pilkar:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.13

Topic: E.06. Posture and Gait

Support: T32GM132498

Title: Peripheral and central factors in age-related difference in balance control

Authors: *H. PARK, A. BAKSHI, J. R. LACKNER, P. DIZIO;
Ashton Graybiel Spatial Orientation Lab., Brandeis Univ., Waltham, MA

Abstract: To study age differences in the interplay among multi-level factors in a balancing task, we used a visual inverted pendulum (VIP) task requiring continuous stabilization of stance-like pendular biomechanics without mechanical loading and postural reflexes, and we simulated altered strength and reflex delay. The participants were 30 younger adults (YA) and 26 older adults (OA). They used a joystick to control an animated beam and bob pivoting about its base. The VIP was programmed as an undamped pendulum (0.27 Hz); the joystick accelerated the pendulum proportionately to its deflection (K_J). Subjects were instructed to keep the VIP at a visible upright reference line (0^o) and avoid visible $\pm 60^o$ "fall" boundaries. When a fall occurred, the VIP was instantly reset to upright and the subject restarted the trial by pressing a thumb button. Every subject experienced all combinations of two joystick gains (K_J =0.025, 0.05) and three delays (0, 0.03, 0.06 s). We analyzed the effects of age and joystick gain and delay on whole-trial variables reflecting overall performance (number of falls, variance of pendulum angular position and velocity) and joystick command strategy (incidence of inactivity, anticipatory adjustments, reactive corrections, and reactive errors driving the pendulum toward a fall). We found significant main effects of age, gain, and delay, as well as an age x gain interaction on the number of falls. There were significantly more falls in OA than YA, in the longer delay conditions, and in the higher gain setting. OA fell significantly more as joystick gain increased but YA did not. No significant age interactions were found for the other variables. We also analyzed periods of imminent falling, where the pendulum was leaning and moving toward a fall boundary, categorized by whether a fall or a successful recovery (crossing the upright) occurred. We compared the incidence of corrective and destabilizing commands for the two outcomes as a function of time before an unavoidable fall. The time of unavoidable falling was defined as the future point where, given the initial velocity, the maximum correct joystick deflection could not prevent reaching the fall boundary. We found significantly more and earlier corrections for recovery than falling, and fewer and earlier destabilizing commands. The differences were significantly larger for YA than OA. The results indicate: 1) a central balancing mechanism compensates for the simulated increases in sensorimotor delays better in YA than OA but equally well to simulated strength changes in YA and OA; 2) a critical, age-dependent error in periods of imminent falling is the decision to move toward or away from the fall boundary.

Disclosures: H. Park: None. **A. Bakshi:** None. **J.R. Lackner:** None. **P. DiZio:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.14

Topic: E.06. Posture and Gait

Support: Natural Sciences and Engineering Research Council

Title: Regional modulation of the ankle plantarflexors during standing perturbations is attenuated following concussion

Authors: J. W. COHEN[1], T. D. IVANOVA[2], *S. GARLAND[3];
[1]Collaborative Program in Musculoskeletal Hlth. Res., [2]Fac. of Hlth. Sci., [3]Western Univ., London, ON, Canada

Abstract: Balance deficiencies are one of the most commonly reported physical symptoms following a concussion. However, the underlying mechanisms of how balance is affected is relatively unknown. In a previous study, we observed that healthy participants respond to directionally induced perturbations by regionally modulating the activity of their ankle plantarflexors. The findings suggested that the central nervous system activates motor units within different regions of the ankle plantarflexors in response to standing perturbations in different directions. Fourteen participants (7 participants within 6 weeks of concussion (return to play stage 3) and 7 age and sex matched healthy controls; mean age 21.8 yrs; 4 females and 3 males per group) participated in an investigation to determine whether the ankle plantarflexor muscles of participants with concussion were modulated according to perturbation direction during unilateral standing perturbations of 1% body mass. High-density surface electromyography (HD-sEMG) was used to determine the amplitude and barycenter of the muscle activation. The barycenter, defined as the weighted mean of the maximal root mean square amplitude across columns and rows, was used to evaluate the medio-lateral (X) and proximo-distal (Y) shifts of muscle activity within the three plantarflexor muscles. In the healthy controls, the HD-sEMG amplitude of the medial gastrocnemius (MG) and lateral gastrocnemius (LG) modulated with the perturbation directions (MG and LG $p < 0.01$, partial $\eta^2 > 0.500$; one-way repeated-measures ANOVAs), however participants with concussion did not modulate the amplitude of their plantarflexor muscles ($p > 0.05$). The barycenter in the LG and the soleus was also modulated across the perturbation directions for healthy controls (LG: $p < 0.01$, partial $\eta^2 = 0.767$ for X coordinates; soleus: $p < 0.01$, partial $\eta^2 = 0.913$ for X&Y coordinates; one-way repeated-measures ANOVAs). No significant modulation of barycenters of all three muscles was observed in participants with concussion ($p > 0.05$) with the magnitude of the barycenter shift being significantly lower in the concussion group compared to healthy controls ($p < 0.05$, Cohen's $d > 1.2$ (values range from 1.2 to 2.9); one-way ANOVA). Further, the relative contribution of MG activation compared to the total gastrocnemii activation was significantly lower in participants with concussion ($p < 0.05$, Cohen's $d = 0.85$; one-way ANOVA). The findings suggest that the responses of the central nervous system are attenuated following concussion at return to play stage 3, and may not be able to meet the demands required for balance during perturbation tasks.

Disclosures: J.W. Cohen: None. **T.D. Ivanova:** None. **S. Garland:** None.

Poster

P563. Posture and Gait: Aging, Injury, and Disease

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P563.15

Topic: E.06. Posture and Gait

Support: NIH Grant EB024570
NIH Grant R01HD098154
Department of the Army W81XWH-16-1-0701

Title: The exchange between task goal variability and energy cost in transfemoral amputee gait

Authors: *I.-C. LEE[1], B. FYLSTRA[1], M. LIU[1], T. LENZI[2], H. HUANG[1];
[1]Biomed. Engin., UNC and NC State, RALEIGH, NC; [2]Mechanical Engineering,, Utah Robotics Center,The Univ. of Utah,, Salt Lake, UT

Abstract: The exchange between task goal variability and energy cost in transfemoral amputee gait

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[b] Utah Robotics Center, Department of Mechanical Engineering, The University of Utah, Salt Lake City, Utah, USA

Minimizing energy costs while walking has been widely accepted for gait regulation. Whereas energy optimization characterizes average gait patterns, there have been rare attempts to reconcile/accommodate gait variability into the explanation. Given that motor redundancy allows individuals to perform tasks reliably while varying in particular ways, to achieve energy optimization, it is of interest to determine how the task goal-irrelevant and goal-relevant variability is regulated. In this study, we investigated how unilateral transfemoral amputees regulate step and stride variability based on the task to achieve energy economy. Nine unilateral transfemoral amputees walked on a treadmill at speeds of 0.6, 0.8, 1.0, 1.2, and 1.4 m/s using their daily passive prosthesis. We applied goal equivalent manifold (GEM) based control to calculate the step-to-step and stride-to-stride variability that were decomposed into goal-relevant and goal-irrelevant manifold during treadmill walking. To quantify the energy economy, the energy recovery rate was calculated based on potential energy and kinetic energy. We found amputees exhibited greater variability along the goal-relevant manifold compared to the goal-irrelevant manifold ($p<.001$) and with less energy recovery rate compared to healthy adults (at least 20% less at faster walking speeds). There was a negative relationship between energy recovery rate and goal-irrelevant variability. Moreover, compared to the standard deviation measurements, the variability decomposed using GEM reflected the amputees' preferred walking speed and the limitation of the passive prosthetic devices. Amputees found a clever way to explicitly exploit the task redundancy and regulate step/ stride variability that was tuned to the goal equivalent manifold. The exploitation of inherent task redundancy allows them to receive potential benefits from motor variability in achieving energy economy. Our findings highlight the limitations of passive prosthetic legs and brings a new perspective motivating the development of powered prosthetic limbs to take into account the task variability allowing

variability distributed along the task space while minimizing variability that interferes with the task goal.

Disclosures: I. Lee: None. **B. Fylstra:** None. **M. Liu:** None. **T. Lenzi:** None. **H. Huang:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.01

Topic: F.04. Stress and the Brain

Title: Probiotics Improve Recovery of Hippocampal Memory Following Disruption from High-Fat Diet

Authors: *S. EL-HULU[1], H. NABI[1], T. J. SCHOENFELD[2];
[2]Psychological Sci., [1]Belmont Univ., Nashville, TN

Abstract: Prolonged high-fat diet (HFD) consumption during adulthood reliably leads to physiological markers of obesity, damage to the hippocampus in the brain, and impairments to spatial memory; however, rescuing these physiological and psychological changes by probiotic therapy is underexplored. Probiotics show some rescue effects of memory impairments, but also do switching back to control diet. Therefore, it is difficult to know whether probiotics, or simply avoiding HFD consumption, is important for restoring good memory as aging progresses. To test this, 16 adult Long-Evans male rats were tested on three different one-trial memory tests: novel object recognition (NOR), novel object location (NOL), and spatial working memory on the radial arm maze (RAM) in a longitudinal design. Performance on each memory test was ascertained at the beginning of the experiment to obtain baseline measurements, following 6 weeks of HFD (60% calories from fat), and weekly during a recovery phase after being switched back to a control and healthy diet. In addition to observing memory changes, metabolic markers of blood leptin and glucose were taken at each time point. To see if probiotic treatment during the recovery phase aided in memory restoration, rats were randomly assigned to two treatment groups following 6 weeks of HFD: the probiotic group (n = 8), syringe-fed diluted Chobani Greek non-fat yogurt daily, and the control group (n = 8), fed the same yogurt but filtered to remove bacterial strains. Results show that HFD significantly impairs memory performance across all rats. During the recovery period, although all rats somewhat improved performance over the course of 4 weeks, the probiotic group had faster recovery, with significantly greater performance across all tests by week 4. Although behavioral recovery was different, blood glucose and leptin levels decreased similarly across both groups during recovery. Together the data suggest that HFD impairs memory performance broadly during adulthood and that return to a healthy diet can rescue these effects. However, pairing with probiotic therapy can hasten the recovery processes, suggesting another brain benefit to the use of probiotics.

Disclosures: S. El-Hulu: None. **H. Nabi:** None. **T.J. Schoenfeld:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.02

Topic: F.04. Stress and the Brain

Title: Acute Exercise Reduces Anxiety and Restores Stress-induced Detriments to Spatial Working Memory

Authors: *J. DAVIS[1], B. MOORE[1], T. J. SCHOENFELD[2];
[2]Psychological Sci., [1]Belmont Univ., Nashville, TN

Abstract: Many rodent studies highlight the impact of chronic aerobic exercise paradigms broadly on cognition, affect, and stress resilience. Despite the common belief that real-time exercise serves as an important stress-coping mechanism in humans, the ability of acute exercise to counteract the effects of stress in rodents has not been explored. In our experiment, adult male Long-Evans rats (n = 8) were habituated for hours at a time to exercise cages containing running wheels to develop a liking towards wheel running without the chronic experience of running. Then, a within-subjects experimental design was used where all rats were tested for spatial working memory on a radial arm maze (RAM) and anxiety-like behavior on an elevated plus-maze (EPM) following either brief exposure (15 minutes) to a running cage or an empty cage after acute music stress (15 minutes) or none. For spatial working memory, acute running did not affect performance at baseline, however acute running did significantly improve performance when preceded by acute music stress ($p < .01$). On the EPM, acute running did reduce anxiety-like behavior at baseline ($p < .01$), but acute music stress prevented these anxiolytic effects. Blood samples were taken to measure corticosterone levels following all four conditions, suggesting that both acute running and music stress increase plasma corticosterone and is unlikely to explain behavioral results. Lastly, effects of acute running alone on optimistic decision-making were examined through the replication of a cognitive bias experiment. Using the RAM, both rough- and smooth-sandpaper textured arms were paired with appetitive or aversively bitter reward, respectively. Once rats learned the sandpaper-reward associations, they were tested on a spectrum of ambiguous sandpaper textures to determine biased behavior in a positive or negative direction, following either brief acute running (15 minutes) or none. It is expected that acute exercise will bias RAM behavior on ambiguous trials in expectation of a positive reward. Overall, results show that rats can be used to model the stress-coping actions of “going for a run” and that brief bouts of running can affect behavior in real time and partially reverse the negative effects of stress on anxiety, performance, and decision-making.

Disclosures: J. Davis: None. **B. Moore:** None. **T.J. Schoenfeld:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.03

Topic: F.04. Stress and the Brain

Title: The Impact of Suppressing Adolescent Hippocampal Neurogenesis on Adult Anxiodepressive Behavior and Stress

Authors: *N. JOHNSON[1], R. LARIVEE[1], T. J. SCHOENFELD[2];
[2]Psychological Sci., [1]Belmont Univ., Nashville, TN

Abstract: Various stressors during the adolescent period can affect development of the brain and have long-lasting impacts on behavior. Specifically, adolescent stress impairs hippocampal neurogenesis and produces increased risk for anxiety, depression, and a dysregulated stress response in adulthood. In order to model the functional effects of reduced hippocampal neurogenesis during adolescence, a transgenic neurogenesis ablation rat model was used to suppress neurogenesis only during the adolescent period and test anxiodepressive behaviors and stress physiology during adulthood, when neurogenesis would be normalized again. Wildtype and GFAP-TK rats (n = 8-9) were given valganciclovir during the first two weeks of adolescence (4-6 weeks old) and injected with bromodeoxyuridine (BrdU) to measure cell proliferation throughout treatment. Starting in young adulthood (13 weeks old), blood was sampled at various time points following acute restraint stress to measure negative feedback of the HPA axis stress response and rats were tested on a battery of anxiodepressive tests (elevated plus-maze, novelty-suppressed feeding, forced swim test, sucrose preference test) at baseline and following acute restraint stress. Hippocampal tissue was additionally analyzed to measure doublecortin in the dentate gyrus to quantify relative neurogenesis during young adulthood and hippocampal volume using Nissl-based stereology to determine long-lasting impacts of neurogenesis arrest during adolescence on hippocampal structure in adulthood. Overall, results show the impact that suppressing neurogenesis has on risk for increased anxiety-like behavior, depressive behavior, and healthy stress physiology and adds further evidence to the dangers of early-life aversive experiences on brain development and behavior.

Disclosures: N. Johnson: None. **R. Larivee:** None. **T.J. Schoenfeld:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.04

Topic: F.04. Stress and the Brain

Support: DGAPA-PAPIIT IN 306 918
PAPIME PE 306 318

Title: Behavioral sex differences after predator scent stress exposure

Authors: *P. TORRES-CARRILLO[1], Y. B. VIDAL-DE LA O[2], D. B. PAZ-TREJO[3], L. D. OCHOA-DE LA PAZ[4], H. SANCHEZ-CASTILLO[5];
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Abstract: The higher prevalence in females to develop stress related disorders could be explain by sex differences in neuroendocrine and behavioral responses to stress, under this explanation, the females have showed more impairment than males, however there is not enough evidence to support that hypothesis. Additionally, the lack of evidence about mechanisms which females had more impairment is explained because most neural and behavioral studies not included females, however, the pattern suggest that stressed males have downregulation on neural activity while stressed females have not alter or have upregulation of neural activity. Such facts support the idea of sex specific stress effects on behavior. The aim of this study was to compare female and male responses to an acute stressor vs. no-stress condition: Predator Scent Stress (PSS) was used as a stress model. Male and female Wistar rats 12 weeks old were used and randomly distributed in a PSS and control group (n=10 per group). Animals of the PSS group were exposed for 10 minutes in an exposure box that contained a bottle with scent tag impregnated with predator urine, whereas the control group only was exposed to the container without the predator urine. Behavior was assessed with novel object recognition (NOR), Barnes maze (BMT), open field (OFT), saccharin preference (SPT), and forced swim (FST) tests 24 hours after the stressor exposure. The results obtained in NOR showed that PSS groups had highest latencies to explore the novel object for the first time. In the BMT's results the PSS induced higher latencies and errors than control groups, but PSS females had more errors than PSS males. Further, in the SPT's results shows that PSS-induced lower preference for saccharin than control groups. Also, PSS females had a higher consumption of liquid in comparison with PSS and control males. Additionally, in FST PSS males increase immobility time than control groups while PSS exposed females decreases. On the other hand, PSS groups exhibited anxiety-like behaviors in OFT. PSS females spend lower time in the central area than PSS males. Also, PSS groups had a lower locomotor activity than control groups. Finally, PSS groups had an increase of time spend immobility and grooming in the OFT. This suggests that PSS induced impairment on memory in females and males but not in the same way. Also, PSS induced anhedonia, a depression-like behavior. Additionally, PSS induced anxiety-like behaviors in OFT. Those impairments could be associated with sex-specific alterations in areas important for learning and memory like the prefrontal cortex and hippocampus and for areas mediating mood and anxiety such amygdala.

Disclosures: P. Torres-Carrillo: None. **Y.B. Vidal-De La O:** None. **D.B. Paz-Trejo:** None. **L.D. Ochoa-de la Paz:** None. **H. Sanchez-Castillo:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.05

Topic: F.04. Stress and the Brain

Support: NIMH IRP

Title: Alpha 2-adrenergic mechanism in stress induced decision-making

Authors: *C. TURNER, V. VISOCKIS, Y. CHUDASAMA;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The α2A-adrenergic receptor is closely associated with stress. In fact, systemic administration of yohimbine, an α2A receptor antagonist increases stress in rats and humans as measured through autonomic physiological responses (Johnston and File, 1989. Pharmacol Biochem. Behav. 32:151-156). In rats, it also leads to impulsive decision-making (e.g., Sun et al. 2010, Biol. Psychiat. 67:649-656). Moreover, the stress response is associated with significant increases in noradrenaline (NA) in the hippocampus (Tanaka et al. 1982. Pharmacol. Biochem. Behav. 16:315-319), and dopamine (DA) in the prefrontal cortex (Thierry et al. 1976. Nature. 263:242-244), which might stimulate the cortex to drive risky behaviors contributing to pathological tendencies towards gambling, substance abuse, and other high-risk behaviors. Here, we explored this possibility by combining a pharmacological stressor, FG7142 with adrenergic agonists and antagonists. We first trained rats on an operant touchscreen decision-making task in which rats chose between two visual stimuli. Responses to the 'safe' stimulus always resulted in the delivery of a certain reward (50µl sucrose). Responses to the 'risky' stimulus resulted in the delivery of an uncertain reward (10µl 75% of the time, and 170µl 25% of the time). The left and right positions of the visual stimuli were determined pseudorandomly. When given a systemic 4 mg dose of FG7142, the rats shifted their baseline strategies by increasing their choice of the safe stimulus and hence the certain reward. In addition, these animals showed long response latencies indicating a slow or deliberated response. In contrast, the co-administration of FG7142 and yohimbine made the rats riskier since these animals not only preferred the option that delivered the uncertain reward but also increased the speed of responding. In comparison, the combination of FG7142 and guanfacine, an α2A receptor agonist, had little impact on the animals' behavior. These data indicate that stress together with acute activation of the noradrenergic system may worsen or trigger risky behaviors observed in both clinical and non-clinical populations. Further studies will examine the combined effects of stress and dopamine stimulation on risky decision-making.

Disclosures: C. Turner: None. **V. Visockis:** None. **Y. Chudasama:** None.

Poster

P615. Stress, Memory, and Cognition

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P615.06

Topic: F.04. Stress and the Brain

Support: Wayne State University Start-up funds

Title: Profiling individual behavioral differences in zebrafish across time using the novel tank test.

Authors: *N. RAJPUT, J. W. KENNEY;
Wayne State Univ., Detroit, MI

Abstract: Social gatherings can be fun for one person, but awkward, for another. Such individual variations in behavior are common, but how they arise is poorly understood. Individuality in behavior is known to be influence by environmental and biological factors. To better understand differences in behavior, we have analyzed zebrafish swim behavior during exploration of a novel tank. When exposed to a novel environment, zebrafish exhibit a variety of exploratory behaviors. To assess these behaviors, we automatically captured three-dimensional swim patterns using depth-sensing cameras. We recorded swim behavior from 400 animals (4-6 months of age) from four commonly used inbred strains (AB, TU, TL, and WIK) and both sexes to study the influence of sex and genetics on behavioral variability. Markerless tracking of the fish was done using a deep learning approach (DeepLabCut) to identify fish posture. To identify postures, we analyzed 200000 video frames. We found that fish posture stratifies into approximately 11 different clusters. Additionally, our analysis of behavioral parameters found that fish behavior stratifies into 5 distinct clusters. Finally, we found that clustering membership is consistent over time and is influenced by strain and sex. With this exploratory study, we provide new insight into the effects of sex and strain on individual behavioral differences in zebrafish.

Disclosures: N. Rajput: None. **J.W. Kenney:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.01

Topic: H.08. Learning and Memory

Support: R01 MH113626
F99 NS119001

Title: Rapid Shifts in the Spectral Dynamics of the Medial Prefrontal Cortex and Hippocampus during Nonspatial Memory for Sequences of Events in Rats

Authors: *A. GARCIA[1], M. JAYACHANDRAN[1], R. P. VERTES[2], T. A. ALLEN[1];
[1]Psychology, Florida Intl. Univ., Miami, FL; [2]FAU/Ctr Complex Systems, Boca Raton, FL

Abstract: A fundamental feature of episodic memory is the ability to remember a sequence of events as they occurred over time, which depends on interactions between the medial prefrontal cortex (mPFC) and the hippocampus (HC; e.g., Jayachandran et al., 2019, *Cell Rep*; Eichenbaum 2017, *NatNeurosci*). While the HC is thought to represent information according to spatiotemporal contexts, mPFC is thought to support the rapid retrieval of information relevant to an action or decision. Nevertheless, how the two structures interact in support of memory for sequences of events is not well understood. Here, we performed simultaneous local field potential (LFP) recordings in mPFC (targeting the prelimbic cortex) and HC (targeting dorsal CA1) using NeuroNexus silicon probes. LFPs in dorsal CA1 have shown to engage theta (5-11Hz) and beta (15-30Hz) during odor sequence memory, with beta activity related to the sequential context (Allen et al. 2016, *JNeurosci*). Here, we expected that mPFC-HC neural dynamics would have complementary spectral modes that reflect the rapid shifting of cognitive processes necessary to perform the sequence memory task. To look at this, we tested five rats (3 females, 2 males) on a sequence memory task with two sequences composed of four unique odors located in nose ports at opposite ends of a linear maze (Side 1, Sequence 1: A_1-B_1-C_1-D_1; Side 2, Sequence 2: A_2-B_2-C_2-D_2). Rats demonstrated sequence memory by holding for >1sec for in sequence odors or withdrawing prior to 1sec for out of sequence odors in order to receive a small water reward. LFPs from mPFC and HC were analyzed using perievent power spectral densities and by looking at coherence between these two regions. We divided our analysis into several epochs including running up to the port, sampling the odor, executing a decision, and receiving the reward. In the spectrograms, we observe strong theta power while running towards the port in HC (and to a lesser degree as the animal poked in the port), strong delta (1-4Hz) power in mPFC around poking behaviors, and strong beta power in both mPFC and HC during odor sampling. Likewise, we observed strong mPFC-HC coherence in all bands, but the task-related modulations were dominated by increased coherence in delta and beta bands during sequence memory. Beta coherence levels seem to be related to performance accuracy, reflecting the olfactory nature of the task. These results indicate that mPFC-HC interactions are highly dynamic and rapidly cycle through multiple modes of rhythmic operation in the delta, theta, and beta bands, reflecting the multiple cognitive stages necessary in memory for sequences of events.

Disclosures: A. Garcia: None. **M. Jayachandran:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.02

Topic: H.08. Learning and Memory

Support: RO1 MH113626
F99 NS119001

Title: Examining Sex and Estrous Cycle in Memory for Sequences of Events in Rats

Authors: *P. LANGIUS[1], M. JAYACHANDRAN[1], F. PAZOS REGO[2], R. P. VERTES[3], T. A. ALLEN[1];
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Abstract: A fundamental feature of episodic memory is the ability to remember a sequence of events as they occurred in time. While rodent studies have examined sex and estrous cycle in spatial and episodic-like memory tasks, little is known about these biological variables in memory for sequences of events. Here, we investigated the role of sex (female/male) and estrous cycle in rats during training and testing phases of an odor sequence memory task (Jayachandran et al., 2019, *Cell Reports*). Rats (10 females, 10 males) were trained on an odor-based sequence memory task composed of two sequences each with four unique odors, delivered in two ports at opposite ends of a linear track. Training occurred over several weeks. First, rats were trained to poke in a nose port and hold for 0-1.2s with an adaptive rule (+10ms for correct trials) for a small water reward on Side1. Second, rats were trained to poke for 1.2s on Side2. Third, time was reduced to 1s, and rats were required to alternate between sides for rewards. Fourth, A_1 and A_2 odors were added to acclimate rats to the odor delivery. Fifth, a second pair of odors were added (Side1, Sequence1: A_1-B_1; Side2, Sequence2: A_2-B_2) forcing rats to poke in the port twice in a row for rewards. Sixth, repeated odors were added (A_1-A_1) which provided a reward for withdrawing prior to 1s. 1s became a decision threshold where rats responded to in sequence odors by holding in the port for >1s and out of sequence odors for <1s. Seventh and eighth, the sequence lengths were extended to three (Side1, Sequence1: A_1-B_1-C_1; Side2, Sequence2: A_2-B_2-C_2) and four odors (Side1, Sequence1: A_1-B_1-C_1-D_1; Side2, Sequence2: A_2-B_2-C_2-D_2). Sequence memory was demonstrated in the last phase when rats responded to both in and out of sequence odors accurately, summarized by a sequence memory index (SMI). Performance across training phases was analyzed by sex and estrous cycle (proestrus, estrus, metestrus, and diestrus) determined by the cellular composition of a vaginal lavage. Results did not show any sex differences in asymptotic sequence memory performance, similar to what we observed in humans performing an analogous task (Reeders et al., 2021, *LearnMem*). Across the estrous

cycle, proestrus and estrus had higher SMI compared to metestrus and diestrus. Some notable observations during training include: females acquired hold times faster; male rats transitioned to new phases faster; female rats had longer pokes, while males had more short pokes (<200 ms). These results indicate that sex differences are evident in training but not testing phase of sequence memory in rats and that the estrous cycle is related to small differences in performance in females during the testing phase.

Disclosures: P. Langius: None. **M. Jayachandran:** None. **F. Pazos Rego:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.03

Topic: H.08. Learning and Memory

Support: Foundation Grant

Title: Laboratory-based Spatial Memory Testing in the Aged Minipig and Domestic Pig

Authors: *L. M. ALLEN[1], M. N. MOUSSA[1], A. DELGADO[1], M. AGUIAR[1], K. LEE[2], T. J. JAROME[3], T. A. ALLEN[1];
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Abstract: Pig (*Sus scrofa*) biology is similar to humans in multiple ways, such as fat distribution, hair coverage, and several organ systems including skin, lung, cardiovascular, metabolic, pancreatic, and gastrointestinal. The pig genome is three times closer to the human genome than that of the mouse (Walters and Prather, 2013, *Advancing Swine Models for Human Health and Diseases*). As such, pigs have been used in translational biomedical research for more than 40 years. However, pigs have rarely been used in behavioral neuroscience, and could benefit translational neurological research on disorders such as age-related cognitive decline and Alzheimer's Disease. As part of a larger strategy in establishing the pig model for translational behavioral neuroscience, we have been developing multiple laboratory-based cognitive/behavioral tasks for pigs. Here we present data from Minnesota minipigs and domestic pigs (mixed-breed) each of which present distinct advantages for neuroscience. For example, the minipig has a more manageable weight across their lifespan, while the domestic pig has a more accessible frontal cortex. Specifically, subjects were minipigs (National Swine Resource and Research Center) or domestic pigs (Wallister Pork) that differed by age (0.5-6.5yrs) to assess viability and differences across cohorts in a classic spatial delayed alternation paradigm. The spatial alternation task is a staple in rodent behavioral neuroscience and responsible for much of

what is known about the neurobiological mechanisms of learning and memory. Spatial memory was tested in a large, fully automated T-maze (17' x 13') with a start box, choice points, automatic rewards (flavored pellets), and return arms separated by guillotine doors. First, we tested the ability for pigs to acquire spatial memory by assessing trials to criteria (3 days at >75% accuracy). We found that younger domestic pigs (n=6, 0.5-1.5yrs) reached criteria quickly over 7 training sessions. By contrast, the older minipigs (n=2, 1.5 & 6.5yrs) demonstrated shallower learning curves: the 1.5yr minipig required 12 training sessions, while the 6.5yr counterpart needed 18 sessions. Next, we tested spatial working memory by introducing various randomly presented delays (5, 60, 120, and 240 sec) which provides a memory decay function. Here, we found that younger pigs performed well declining from 95-63% accurate across delays, while aged minipigs had a slight decrease in overall performance, but similar decay rate from 85-60% accurate across delays. We are continuing to test aged minipigs in order to see if spatial memory differences vary as a function of age, or reflect other cohort differences.

Disclosures: L.M. Allen: None. **M.N. Moussa:** None. **A. Delgado:** None. **M. Aguiar:** None. **K. Lee:** None. **T.J. Jarome:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.04

Topic: H.08. Learning and Memory

Support: R01 MH113626
F99 NS119001

Title: Neuronal Representations in Medial Prefrontal Cortex during Nonspatial Memory for Sequences of Events

Authors: *M. JAYACHANDRAN[1], A. VASALLO VELIZ[1], S. LEYVA[1], R. P. VERTES[2], T. A. ALLEN[1];
[1]Psychology, Florida Intl. Univ., Miami, FL; [2]FAU/Ctr Complex Systems, Boca Raton, FL

Abstract: Memory for sequences of events facilitates organization of episodic experiences into serial or ordinal associations representing the flow of events that have occurred. It is established that hippocampus (HC) is essential for formation (e.g., Fortin et al., 2002, *NatNeurosci*) and representation of sequential context (Allen et al., 2016, *JNeurosci)*. We recently showed that specific cell populations in medial prefrontal cortex (mPFC) differentially contribute to retrieval strategies in sequence memory (Jayachandran et al., 2019, *Cell Rep*). Yet, how individual mPFC neurons respond while processing sequence memory is not fully understood. We expect mPFC activity to be related to the sequential context, ordinal position, and accuracy aspects of the task.

Specifically, mPFC neurons should encode features with a temporal pattern complementing CA1 dynamics. Here, rats were trained to remember two sets of four odor sequences (Side 1, Sequence 1: A_1-B_1-C_1-D_1; Side 2, Sequence 2: A_2-B_2-C_2-D_2) presented at opposite ends of a linear track. Rats demonstrated sequence memory in both sequences by holding their nose in the port for >1s for in sequence odors, and withdrawing prior to 1s for out of sequence odors for a water reward. We recorded ~200 neurons during the task in five rats (2 males, 3 females) using NeuroNexus silicon probes with a 2x4 tetrode pattern targeting prelimbic cortex and dorsal CA1. Single mPFC neurons were isolated offline, and their spike times analyzed across task variables (odors, positions, sequential context, and accuracy) in temporal analysis windows aligned to poking behaviors (poke in/out). Results showed that ~30% of mPFC neurons were related to position, sequential context and accuracy, but not odors. Moreover, mPFC spike activity changed around poking behaviors with ramping-like changes in firing rates. These response patterns generalized between sequences, and were thus nonspatial. In addition, local field potential dynamics reliably show a patterned dominance of HC theta (5-11Hz) running up to the port, mPFC delta (1-4Hz) around poking behaviors, and mPFC and HC coherent beta (15-30Hz) during odor sampling. We next looked within spike-phase relationships in these spectral bands and observed mPFC spikes are often entrained to local delta around poking behaviors, and phase entrained to beta and theta related to trial accuracy (Symanski et al., 2021, *bioRxiv*). The spiking dynamics raise interesting questions about the supremacy of different rhythmic mechanisms governing mPFC neuronal integration during processing epochs related to sequence memory. Overall, the results are consistent with sequence memory as a mPFC-HC based process.

Disclosures: M. Jayachandran: None. **A. Vasallo Veliz:** None. **S. Leyva:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.05

Topic: H.08. Learning and Memory

Support: NIH MH113626
FIU CASE Distinguished Postdoctoral Program

Title: Optogenetic stimulation of nucleus reuniens of the thalamus elicits frequency and projection specific changes in medial prefrontal cortex and ventral hippocampus activity

Authors: T. D. VIENA[1], B. SETLOW[2], F. D. LUBIN[3], R. P. VERTES[4], T. A. ALLEN[1]; [1]Dept. of Psychology, Florida Intl. Univ., Miami, FL; [2]Dept. of Psychiatry, Univ. of Florida, Gainesville, FL; [3]Dept. Neurobiol, Univ. Alabama Birmingham, Birmingham, AL; [4]Ctr. for Complex Systems and Brain Sci., Florida Atlantic Univ., Boca Raton, FL

Abstract: Abnormal synchronization in the prefrontal-hippocampal circuit is a hallmark of several neuropsychiatric disorders. Anatomically, nucleus reuniens (RE) projects to both the medial PFC (mPFC) and hippocampus (HC), including a population of neurons that simultaneously project to both structures (Viena et al., 2020, *Hippocampus*). Previous work has demonstrated the involvement of RE in cognitive tasks that require mPFC-HC interactions, and importantly, involvement in the rhythmic activity of the mPFC-HC system in the theta (5-11Hz) and delta bands (1-4Hz; Dolleman-van der Weel et al., 2019, *LearnMem*). One current hypothesis is that RE is responsible, in part, for mediating coordinated interactions within the mPFC-HC network in support of cognition. It is unclear, however, which RE neurons are capable of modulating mPFC-HC rhythms. We tested this capacity using an optogenetics/AAV-retrograde strategy to differentially activate RE neurons that project to mPFC or ventral CA1. Rats received bilateral retrograde AAV injections of channelrhodopsin (ChR2) in prelimbic cortex (n=5) or vCA1(n=4), while control rats received non-ChR2 injections (n=5). All rats were implanted with stainless-steel electrodes bilaterally in mPFC and vCA1 to record local-field potential (LFP) and unit activity, and an optrode above RE for LED-based blue light stimulations. Recordings occurred in an open field maze during free behavior. ChR2 infected RE neurons were stimulated with square pulses or sine waves at frequencies known to be intrinsic to RE neurons (Viena et al., 2021, *BehavBrainRes*; 1, 2, 4, 8, 20, 50Hz; 5 min blocks each). When stimulated at these frequencies, the resultant LFP rhythm included the stimulated frequency superimposed on the intrinsic activity of experimental rats but not in controls. The strongest effects were in pathway targeted regions (i.e., RE⇒mPFC or RE⇒vCA1). This effect was present in mPFC and vHC when we applied sine waves, but it was weaker or absent with pulses. Compared to controls, overall delta rhythms were enhanced in vHC, and overall theta rhythms were enhanced in mPFC. Consistent with glutamatergic projections from RE, we observed single neurons in downstream targeted areas (mPFC) that were directly activated by blue light activation of ChR2 in RE. In combination, findings demonstrate that activation of RE⇒mPFC or RE⇒vCA1 pathways can elicit frequency and projection-specific changes in mPFC and vHC activity. Our findings support the idea that RE influences the activity of mPFC-HC system, potentially by sustaining synchronous changes in these target regions to support higher-order cognitive processes associated with mPFC-HC interactions.

Disclosures: T.D. Viena: None. **B. Setlow:** None. **F.D. Lubin:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.06

Topic: H.08. Learning and Memory

Support: NIH Grant MH113626
FIU CASE Distinguished Postdoctoral Program

Title: Dual medial prefrontal cortex and hippocampus projecting neurons in the paraventricular nucleus of the thalamus of rat

Authors: *G. E. RASCH, T. D. VIENA, T. A. ALLEN;
Cognitive Neurosci., Florida Intl. Univ., Miami, FL

Abstract: The paraventricular nucleus (PVT) of the midline thalamus is a critical integration site and contributes to emotional memory, context learning, reward seeking, and feeding behavior (e.g., Do-Monte et al., 2015, *Nature*). The diversity of its functions is supported by its anatomical connections to both cortical and subcortical regions, including medial prefrontal cortex (mPFC), central/basal nuclei of amygdala, and hippocampus (HC) (Vertes & Hoover, 2008, *Comparative Neurology*). While studies have shown that PVT neurons project to mPFC and HC separately, it is not known whether these come from the same or different cell populations. Previous investigations show single and dual mPFC and HC projecting neurons in the nucleus reuniens (RE) (Viena et al, 2020, *Hippocampus*). Using a dual retrograde adeno-associated viral approach, we sought to identify neuronal populations within PVT that project to medial prefrontal cortex (mPFC), ventral HC (vHC) and/or neurons with collaterals to both regions. Simultaneous bilateral injections were delivered targeting mPFC deep layers V/VI (0.4μL of AAVr-CAG-TdTomato) and ventral CA1 regions of vHC (0.5 μL of AAVr-CAG-GFP) in four rats. Quantification of retrogradely labeled neurons was performed in four levels throughout the anteroposterior axis of PVT. Results showed that PVT contains a population of neurons that simultaneously project to mPFC and vHC (~2%), with no significant differences in the prevalence of this population across the anteroposterior axis. Next, we examined the calcium binding protein expression in these cells. We previously showed that the distribution of calcium binding proteins calretinin (CR) and calbindin (CB) cells segregate throughout the nuclei of the midline thalamus including PVT. We found that dual mPFC-vHC projecting cells in PVT did not express either CR nor CB, however, CR^+ and CB^+ immunopositive PVT cells were spatially segregated into random clusters surrounding dual projecting PVT cells. Generally, dual mPFC-vHC projecting cells in PVT share the characteristics found in RE. This newly identified PVT cell population that projects to both mPFC and vHC forms a unique circuit that may, like RE, may provide a synchronization mechanism for coordinating mPFC-vHC activity but for cognitive functions more associated with PVT.

Disclosures: G.E. Rasch: None. **T.D. Viena:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.07

Topic: H.08. Learning and Memory

Support: R01MH113626
R01ES006189

Title: Reducing Seizure and Behavior-Related Hypersynchrony of the Hippocampal Theta Rhythm Caused by Chronic Exposure to Lead (Pb^{2+})

Authors: *N. W. SCHULTHEISS[1], J. L. MCGLOTHAN DZIEDZIC[2], T. R. GUILARTE[2], T. A. ALLEN[3];
[2]Envrn. Hlth. Sci., [3]Dept. of Psychology, [1]Florida Intl. Univ., Miami, FL

Abstract: Pb^{2+} is a NMDA receptor antagonist, and exposure during brain development can cause permanent intellectual impairments and social, emotional, and behavioral dysregulations. Pb^{2+} exposure is also a risk factor for schizophrenia (SZ) and epilepsy (Epi). Pb^{2+} neurotoxicity exhibits significant comorbidity SZ and Epi; biomarkers for Pb^{2+} exposure correlate to SZ incidence and symptom severity; and Pb^{2+} exposure exacerbates seizures and worsens cognitive and sensorimotor deficits in experimental models of SZ. Childhood Pb^{2+} exposure reduces parvalbumin-positive GABAergic interneurons (PVGIs) in prefrontal cortex (PFC) and hippocampus (HC), a hallmark of schizophrenia. These fast-spiking PVGIs are integral to thetarhythmic HC synchronization (6-10 Hz), that in turn coordinates gammafrequency neuronal interactions (25-120 Hz). As we have recently demonstrated, developmental Pb^{2+} exposure causes hypersynchrony of the HC theta rhythm and gamma oscillations, potentially arresting functional coupling between HC and PFC. Pb^{2+} exposure also increased absence seizures marked by synchronous discharges with a theta fundamental frequency (Schultheiss et al., 2020, bioRxiv 181149). It is a provocative possibility that cortical SWDs entrain intrinsic HC mechanisms of rhythmogenesis, contributing to learning and memory deficits. At present there are no effective treatment strategies for theta hypersynchrony.We analyzed local field potential recordings (LFPs) from control and Pb^{2+}-exposed rats (Schultheiss et al. 2020b, BehavNeurosci) to test whether 7,8dihydorxyflavone (DHF), a TrkB-mimetic and common dietary supplement, can ameliorate theta hypersynchrony. Following baseline sessions, rats were given daily DHF injections (5 mg/kg) for 2 weeks. After controlling for locomotion, theta was normalized to DHF(-) values in control rats. Distributions of theta power for Pb^{2+}-exposed rats showed that DHF markedly reduced theta hypersynchrony ($t(286) = 10.64$, $p = 1.68x10^{-22}$), and a 2 x 2 ANOVA revealed a significant interaction of Pb^{2+} x DHF ($F(1,608)=20.0$, $p=9.1x10^{-6}$). This suggests a possible avenue to improve neurocognitive function in millions of people using a safe, inexpensive supplement. We also found that absence seizures were abolished by the antiepileptic drug ethosuximide (50-200 mg/kg). Complementing DHF, blocking absence seizures in children could reduce neurotoxic effects of Pb^{2+}. We propose that developmental Pb^{2+} neurotoxicity shares pathophysiological mechanisms with SZ and absence epilepsy, defining a distinct neurophysiological endophenotype underlying similar patterns of cognitive impairment across conditions.

Disclosures: N.W. Schultheiss: None. **J.L. McGlothan Dziedzic:** None. **T.R. Guilarte:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.08

Topic: H.08. Learning and Memory

Support: R01 MH113626

Title: Dual-projecting cells linking thalamic and cortical communication routes between the medial prefrontal cortex and hippocampus

Authors: *M. SCHLECHT, M. JAYACHANDRAN, G. E. RASCH, T. A. ALLEN; Florida Intl. Univ., Miami, FL

Abstract: Interactions between the agranular medial prefrontal cortex (mPFC) and hippocampus (HC) play a major role in basic cognitive and behavioral functions, especially those related to memory-based decision making (e.g., Dolleman-van der Weel, 2019, *LearnMem;* Eichenbaum, 2017, *NatNeurosci*). Dysfunction in mPFC-HC have been specifically implicated in several neurological disorders including schizophrenia, epilepsy, frontotemporal dementia, and Alzheimer's disease. Although HC projects directly to mPFC, return projections go through an intermediary region. In memory, the ventral midline thalamus (vmThal) and parahippocampal cortex constitute major communication pathways in mPFC-HC interactions. These thalamic and cortical routes of communication between mPFC and HC provides the interface for executive functions, working memory, and long-term memory integration. While the anatomy of the thalamic and cortical pathways in the mPFC-HC circuitry have been explored in detail elsewhere, it is not known about whether the afferent regions shared by vmThal, lateral entorhinal cortex (LEC), and perirhinal cortex (PER) arise from the same or different (non-overlapping) cell populations. Here, we sought to determine whether these two regions share cell-specific inputs that could activate patterns in both routes simultaneously. To do this we used a dual fluorescent retrograde tracing approach using cholera toxin subunit-B conjugated with Alexa Fluorophores (CTB-488 and CTB-594) with injections targeting vmThal (0.3 µL of CTB) and the parahippocampal region (LEC/PER; 0.5 µL of CTB) in rats. Retrograde cell body labeling was examined in key regions of interest within the mPFC-HC system including: (1) mPFC, specifically anterior cingulate cortex (ACC), dorsal and ventral prelimbic cortex (dPL, vPL), and infralimbic cortex (IL); (2) medial and lateral septum (MS, LSd, LSi, LSv); (3) subiculum (Sub) along the dorsal-ventral and proximal-distal axes; and (4) medial and lateral entorhinal cortex (MEC, LEC). Results showed that dual vmThal-LEC/PER-projecting cell populations are found in MS, ventral Sub, and the shallow layers II/III of MEC and LEC.

Interestingly, we did not find any dual vmThal-LEC/PER-projecting cells in mPFC or in the cornu ammonis (CA) subfields of the HC. Therefore, mPFC and HC activity is sent to vmThal and parahippocampal cortex via non-overlapping projection cell populations from HC proper. These newly described dual vmThal-LEC/PER-projecting cell populations in MS, ventral Sub, and LEC are in a unique position to simultaneously influence both the cortical and thalamic mPFC-HC pathways.

Disclosures: M. Schlecht: None. **M. Jayachandran:** None. **G.E. Rasch:** None. **T.A. Allen:** None.

Poster

P825. Thalamic and Brainstem Circuits I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P825.09

Topic: H.08. Learning and Memory

Title: Locating the midline thalamus in humans using diffusion weighted imaging for the investigation of neurological disorders

Authors: *P. C. REEDERS[1], A. T. MATTFELD[1], T. A. ALLEN[1,3,2];
[1]Dept. of Psychology, [2]Dept. of Envrn. Hlth. Sci., Florida Intl. Univ., Miami, FL; [3]Ctr. for Complex Systems and Brain Sci., Florida Atlantic Univ., Boca Raton, FL

Abstract: The midline thalamus coordinates activity in higher-order cortico-thalamic-cortical networks, most notably those that involve the agranular medial prefrontal cortex and medial temporal lobe. At a basic level, the midline thalamus is critical to cognition at the intersection of executive and memory function. At the clinical level, midline thalamic pathology is associated with several neurological disorders including Alzheimer's disease, epilepsy, drug addiction, mood disorders, and others. In Alzheimer's disease (AD), neurofibrillary tangles are present in the thalamus, which target the midline nuclei, when symptoms are most severe (Braak & Braak, 1991, *Acta Neuropathol*, p. 261-268). Based on specific patterns of anatomical connectivity in rodents and macaques, four midline thalamic nuclei have been identified including paratenial nucleus (PT), paraventricular nucleus (PV), rhomboid nucleus (RH), and the nucleus reuniens (RE). However, almost nothing is known about the midline thalamic nuclei in humans *in vivo*. This is due, in part, to the difficulty in identifying the limbic thalamus with current imaging technologies. Here, we sought to identify the midline thalamus *in vivo* by using probabilistic tractography and *k*-means clustering with diffusion weighted imaging data. Thalamic nuclei were clustered and sorted based on connectivity profiles (cortical and subcortical targets). This approach revealed a midline thalamic cluster that is well connected to the agranular medial prefrontal cortex and medial temporal lobe and located directly adjacent the third ventricle. This pattern was identified as the human midline thalamus. We created two masks for human midline

thalamus dividing a dorsal midline thalamic mask, which includes the PV and PT, from a ventral midline thalamic mask, which includes the RH and RE. These divisions are important because dorsal and ventral midline thalamus are known to contribute to different cognitive functions in rodents. Ultimately, this connectivity-based identification and segmentation method can be used to help characterize neurological brain pathologies by investigating alterations in the quantified structural connectivity between the midline thalamus, medial temporal lobe, and medial prefrontal cortex region. We are currently examining these patterns across stages of Alzheimer's disease.

Disclosures: P.C. Reeders: None. **A.T. Mattfeld:** None. **T.A. Allen:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01NS113804

Title: Theta synchronized stimulation increases hippocampal excitability in humans

Authors: *J. E. KRAGEL[1], S. M. LURIE[1], M. J. SCHATZA[4], E. B. BLACKWOOD[4], E. A. CHUNG[1], C. ZELANO[2], S. U. SCHUELE[2], J. F. DISTERHOFT[3], A. S. WIDGE[4], J. L. VOSS[1,2];
[1]Med. Social Sci., [2]Neurol., [3]Physiol., Northwestern Univ., Chicago, IL; [4]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: Theta oscillations organize memory processing across cortico-hippocampal networks to support memory function. The phase of theta dictates the flow of information to and from the hippocampus, with sensory inputs optimally timed to arrive at the trough of hippocampal theta. Modulation of hippocampal activity timed to the ongoing theta rhythm would provide a framework to causally assess a behavioral role of theta and develop stimulation-based techniques to restore memory function. However, phase-locked stimulation of hippocampal networks and resulting neurophysiological changes have not been characterized in humans. Here, we used a phase-locking algorithm to drive direct electrical stimulation (DES) of the lateral temporal cortex, a node of the hippocampal network. Network-targeted stimulation was timed to the trough of invasively recorded hippocampal theta oscillations (N=2 participants). After 30 minutes of phase-locked DES, evoked potentials in the hippocampus increased in amplitude (both $p = .01$, permutation tests). In addition, we assessed changes in synchronization between the hippocampus and the stimulation site using the debiased weighted phase lag index. Phase-locked stimulation increased theta synchrony (4 - 8 Hz, participant one, $p = .01$; participant two,

p = .04, permutation tests) during rest. Taken together, these preliminary findings suggest that theta-phase synchronized stimulation is an effective means to modulate hippocampal excitability and network strength, providing a pathway to modulate hippocampal-dependent behaviors.

Disclosures: J.E. Kragel: None. **S.M. Lurie:** None. **M.J. Schatza:** None. **E.B. Blackwood:** None. **E.A. Chung:** None. **C. Zelano:** None. **S.U. Schuele:** None. **J.F. Disterhoft:** None. **A.S. Widge:** None. **J.L. Voss:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.02

Topic: H.08. Learning and Memory

Support: NIH Grant R01 NS113804

Title: Effects of closed-loop phase-locked stimulation on cortico-hippocampal connectivity in rats

Authors: *E. SONG[1], A. ALPERS[1], K. WARNER[1], M. SCHATZA[1], C. WEISS[2], H. S. WIRTSHAFTER[2], J. F. DISTERHOFT[2], J. L. VOSS[3], A. S. WIDGE[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Northwestern Univ. - Chicago, Chicago, IL; [3]Med. Social Sci., Northwestern Univ., Chicago, IL

Abstract: The hippocampus (HPC) and its interactions with broader brain networks are essential for memory processing. In rats, temporal association cortex area 2 (TE2), a region that is dorsally adjacent to the perirhinal cortex, communicates with the HPC and plays an important role in recognition memory. Synchronization of local field potentials (LFP) has been linked with network functions throughout the brain. Here we report that a novel phase-locked stimulation algorithm developed by our group can alter LFP synchronization within the hippocampal network. The effects of the phase-locked stimulation were examined in three ways: 1) TE2->HPC synchrony, 2) synchrony among HPC subfields, and 3) acute and cumulative effects of multiple daily sessions. Long Evans rats were implanted with a 32-channel silicon probe in the dorsal hippocampus and an 8-channel microwire bundle with a stimulating wire in TE2. Neural signal was recorded while rats were behaving in an open field and performing a touch screen memory task in an operant chamber. We demonstrated that phase-locked stimulations in TE2, locked to specific stages of the theta/alpha oscillation in the hippocampus, could increase or decrease the coherence in the theta-alpha band throughout the network. Specifically, 100µA 90µs biphasic pulses delivered to TE2 at 0º or 90º phases of a hippocampal 6Hz oscillation increased and decreased, respectively, the TE2-HPC coherence. The efficacy of different current intensities, phase degrees and oscillation frequencies were also examined. Our results suggest

that a single 30min phase-locked stimulation session could induce changes in coherence and that a 30min session of stimulation daily over the course of 2-3 weeks could potentially lead to sustained changes in coherence between the TE2 and the HPC. The data also support that the synchronization of the subfields within the HPC can be altered following closed-loop stimulation. For instance, the coherence between the dentate gyrus and the CA1 was increased after stimulation. Additionally, we measured effects of stimulation on memory using a spatial object recognition memory task (paired associate learning). Behavioral effects of the phase-locked stimulation are currently being evaluated. This study, to our best knowledge, is the first to demonstrate that closed loop stimulation can causally manipulate frequency-specific coherence in hippocampal networks and thus potentially improve learning and memory. The present work provides important insights and therapeutic potential in treating patients with memory disorders.

Disclosures: E. Song: None. **A. Alpers:** None. **K. Warner:** None. **M. Schatza:** None. **C. Weiss:** None. **H.S. Wirtshafter:** None. **J.F. Disterhoft:** None. **J.L. Voss:** None. **A.S. Widge:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.03

Topic: H.08. Learning and Memory

Support: NIH Grant NS113804

Title: Hippocampal potentials evoked by network-targeted stimulation vary by theta phase

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Abstract: Although stimulation targeting the hippocampus indirectly via its network can impact hippocampal memory function, the mechanisms governing its effects are not fully understood. For example, though CA3 excitability and entorhinal connectivity vary with hippocampal theta phase (Hasselmo et al. 2002), it is not known whether phase has an impact on network stimulation efficacy. To address this question, we tested whether corticocortical evoked potential (CCEP) amplitude in the human hippocampus varies according to local theta phase at the time of stimulation. Intracranial local field potential data were collected from individuals with epilepsy undergoing medically necessary stereotactic electroencephalographic recording (sEEG) (n = 7 participants, 23 hippocampal sEEG contacts). Biphasic bipolar direct electrical stimulation (1Hz, 5mA) was delivered via sEEG contacts in the lateral-temporal node of the hippocampal network.

Lateral-temporal stimulation consistently yielded CCEPs in ipsilateral hippocampus with distinct early (N1) and late (N2) negative components. We hypothesized that N1 and N2 amplitudes would vary periodically with local theta phase at stimulation onset. Further, we hypothesized that stimulation would evoke maximum component amplitude when delivered at theta trough and minimum component amplitude at peak. After estimating theta phase at stimulation for each trial and electrode, we performed circular-linear analysis to determine if component amplitude varied continuously according to phase. There was significant circular-linear correlation between theta phase and mean amplitude during both N1 ($P<0.002$) and N2 ($P<0.01$; computed via permutation testing). Control analyses on stimulation-free epochs indicated that the periodic stimulation effects were not merely due to a relationship between phase and future amplitude. Contrary to our hypothesis, N2 amplitude was significantly greater in peak trials than trough trials, an effect which persisted even after correcting for the stimulation-free amplitude predicted by phase at stimulation onset ($P<0.02$). These findings suggest that local theta phase predicts the magnitude of evoked hippocampal response by stimulation of lateral temporal afferents. Comparative analyses of phase-based variation in hippocampal responses to network-targeted stimulation in the Long-Evans rat will also be discussed (see also Song et al., SFN 2021). These findings are relevant to understanding mechanisms for control of hippocampal function via network-targeted stimulation.

Disclosures: S.M. Lurie: None. **J.E. Kragel:** None. **E. Song:** None. **M. Schatza:** None. **S.U. Schuele:** None. **J.F. Disterhoft:** None. **A.S. Widge:** None. **J.L. Voss:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.04

Topic: H.08. Learning and Memory

Support: NIH R01 NS113804

Title: Functional connectivity between the temporal association cortical area 2 and the hippocampus in rats

Authors: *A. ALPERS[1], E. SONG[1], K. WARNER[1], C. WEISS[2], J. DISTERHOFT[2], J. L. VOSS[3], A. S. WIDGE[1];
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Abstract: Temporal association cortical area 2 (TE2) in rats is homologous to the lateral temporal cortex in humans and is implicated in episodic memory functions. Human lateral cortex has been used as a location for network-targeted stimulation to indirectly influence the

hippocampus. Thus, mapping connectivity of TE2 to hippocampus in rats is important for developing a rodent model for human network-targeted stimulation. TE2 is known to connect to the perirhinal cortex, but its exact connections to the hippocampus are not well known. The objective of this study was to determine if TE2 in rats is functionally connected to the hippocampus, and if so, where. We electrically stimulated TE2 in one hemisphere in Long Evans rats (n=6, 3M/3F) using 0.1 ms biphasic (300 µa) pulses at a frequency of 10 Hz for four minutes, then stained slices for the immediate early gene *c-fos* 60 min following the stimulation. We found that stimulation in area TE2 leads to strong *c-fos* activation in the dentate gyrus (DG) throughout the length of the hippocampus, both dorsally and ventrally. This expression starts from -2.16 AP behind bregma to -6.12 AP. In addition, we found *c-fos* activation in the dorsal and ventral CA1 and CA3 subfields, though it did not fluoresce as brightly as in the DG. This evidence of connectivity was consistent in the hemisphere that was stimulated, but not in the contralateral hemisphere. We also recorded neural data from the hippocampus (both DG and CA1) at -5.3 AP while electrically stimulating TE2 and found low-frequency activity synchrony between TE2 and these areas. Altogether, this evidence suggests that area TE2 in the rat is ipsilaterally connected to the dentate gyrus, CA1, and CA3 hippocampal subfields. We posit, based on previous studies as well as preliminary analysis of the timing of stimulation data, that TE2 connects first through the perirhinal and entorhinal cortex, then to the hippocampal subfields starting with DG and then to CA1 and CA3. This has important implications in studies of the memory system, especially as TE2/temporal cortex is well positioned as a target to noninvasively modulate the hippocampus in human patients.

Disclosures: A. Alpers: None. **E. Song:** None. **K. Warner:** None. **C. Weiss:** None. **J. Disterhoft:** None. **J.L. Voss:** None. **A.S. Widge:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.05

Topic: H.08. Learning and Memory

Support: NIH Grant RO1NS113804
NIH Grant R25GM121231

Title: Rats learn multiple sets of visual discriminations during paired associate learning

Authors: *C. WEISS[1], E. SONG[3], M. M. OH[1], S. M. LURIE[2], M. J. SCHATZA[3], A. GALVEZ[1], A. S. WIDGE[3], J. L. VOSS[2], J. F. DISTERHOFT[1];
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Abstract: Hippocampal memory processing can be influenced via network-targeted stimulation to locations of the broader hippocampal network, providing indirect impact on hippocampal activity and associated memory processing. To understand the physiological basis of such effects we use rats to examine different stimulation protocols, e.g. we report in this session that stimulation of temporal Area TE2 phase-locked to the LFP at 0^{o} significantly increases coherence of TE2-hippocampal activities. We validated a paired-associate learning (PAL) task using the Bussey-Saksida touch-screen apparatus. We use a Latin square design to test four different stimulation patterns. This requires four sets of unique stimuli to test the effects of four different stimulation procedures. Given that most studies use only one set of visual stimuli we tested the responsivity of young adult Long Evans rats to four different sets of visual stimuli. The following sets were used (shown are the rewarded positions planned for each stimulus; during any trial one correct, one blank, and one incorrect image/position pairs are presented among the three panels of the touchscreen). Rats were first trained to respond to images in order to retrieve a sucrose pellet reward. Most rats (9 of 12 in cohort 1; 6 of 8 in cohort 2) gradually increased their percentage of correct responses during the different training phases. Rats that were slow to master the initial steps of training continued to have trouble on each successive step. A threshold of 7 sessions per training step differentiated learners from slow learners. After the pairwise discrimination (PD) task, a pilot group of 7 rats continued PD training using stimuli from either Set 3 or Set 4. Rats increased their responding (GT 60 trials) and accuracy (GT 60%) for both sets over 4 sessions, i.e. the new stimuli were differentiated by the rats. No obvious sex differences in responding were noted. These findings will be useful for experiments that involve testing multiple interventions on episodic-like memory, including for our own experiments using phase-locked stimulation.



Disclosures: C. Weiss: None. **E. Song:** None. **M.M. Oh:** None. **S.M. Lurie:** None. **M.J. Schatza:** None. **A. Galvez:** None. **A.S. Widge:** None. **J.L. Voss:** None. **J.F. Disterhoft:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.06

Topic: H.08. Learning and Memory

Support: NIH R01MH111439
NIH P50MH109429

Title: Examining hippocampal sharp-wave ripple-locked activation in language networks

Authors: *A. MISHRA[1,2], S. AKKOL[1,2], N. MARKOWITZ[1,2], E. ESPINAL[1,2], A. D. MEHTA[2,1], S. BICKEL[1,2,3];
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Abstract: Hippocampal sharp-wave ripples (SWR) may serve as a conductor of cortical regions to foster the encoding and retrieval of stored memories. Prior studies showed SWR locked cortical activation in a variety of tasks, but their involvement in reactivating cortical language networks is unknown. To investigate this, we employed a list-learning task where participants were presented with twelve semantically unrelated words, structured in either a set of three sentences or in a randomized word list. After a mathematical distractor task, participants were asked to verbally recall words that they remembered. We obtained intracranial EEG recordings in four patients undergoing treatment for intractable epilepsy that graciously volunteered their time. We identified candidate hippocampal SWR by filtering in a 70-180Hz ripple band before isolating SWR that showed correct physiological form. We found that SWR activity peaked after the onset of verbal response in the recall portion of the task ($p < 0.05$ for 800ms after recall onset). To examine reactivation in the language network, we then isolated electrodes that exhibited selective increase of high-frequency activity (HFA, 60-160Hz) during listening to the sentence trials (n = 55 contacts), word list trials (n = 53), or non-selective activation in both (n = 76). During recollection of words, the identified language regions exhibited response ripple-locked reactivation in preferred recall trials (sentence selective contacts in recall sentence trials, randomized word areas in recall randomized word trials, significant time-bins from -50 to 150ms). Further, we found a sustained HFA increase following SWRs in both conditions in the lateral anterior temporal cortical regions (n = 218) which correlated with HFA activation in all language-responsive areas. Our results extend previous findings from the visual domain to the language network in that task specific cortical networks show co- (or re-) activation with hippocampal SWRs.

Disclosures: A. Mishra: None. **S. Akkol:** None. **N. Markowitz:** None. **E. Espinal:** None. **A.D. Mehta:** None. **S. Bickel:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.07

Topic: H.08. Learning and Memory

Support: NINDS R21NS095094

Title: Theta-gamma phase-amplitude coupling and the fMRI BOLD signal

Authors: *P. F. HILL[1], D. X. WANG[2], M. D. RUGG[3], A. D. EKSTROM[1], D. R. KING[3], B. C. LEGA[4];
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Abstract: Local changes in the fMRI blood-oxygen-level dependent (BOLD) signal are typically equated with commensurate changes in gamma-band activity. However, the electrophysiological dynamics underlying inter-regional BOLD connectivity remain under explored. Phase-amplitude coupling (PAC) has been advanced as a candidate mechanism for coordinating local brain activity across different spatial scales. We examined whether the strength of task-evoked cortico-hippocampal phase coherence and theta-gamma PAC differentially predict variance in functional BOLD connectivity between the same cortico-hippocampal ROIs. Fifteen neurosurgical patients implanted with depth electrodes performed a verbal free recall task while electrophysiological activity was recorded simultaneously from hippocampal and neocortical sites. The same patients performed a similar version of the task during a later fMRI session. Local and cross-regional subsequent memory effects were computed for both imaging modalities as patterns of brain activity predictive of later free recall. Cross-regional phase coherence and PAC between pairs of cortico-hippocampal contacts was computed for theta [slow (2-4 Hz), fast (4-8 Hz)] and gamma [low (30-70 Hz), high (70-150 Hz)] frequencies and contrasted according to subsequent memory status. BOLD connectivity was computed between the same cortico-hippocampal ROIs as the contrast between beta-series correlations for subsequently remembered and forgotten study items. Linear-mixed effects modeling revealed that intra-regional measures of theta-gamma PAC predicted significant variance in neocortical BOLD activity independently of local increases in theta and gamma power. Estimates of cross-regional phase coherence between slow theta oscillations measured in the hippocampus and lateral prefrontal cortex predicted significant variance in BOLD connectivity between the same regions. By contrast, cross-regional PAC measured between hippocampal theta and cortical gamma did not reliably co-vary with BOLD connectivity between cortico-hippocampal ROIs. Follow-up analyses confirmed that the relationship between cross-regional PAC and BOLD connectivity was not moderated by hemisphere, long axis location of the hippocampal seed, lobar location of the cortical target, or the direction of task-evoked BOLD connectivity. Taken together, these results suggest that theta-gamma PAC contributes to local measures of BOLD activity but does not provide a reliable index of cross-regional BOLD connectivity. This has important implications for interpreting cortico-hippocampal interactions obtained from different imaging modalities.

Disclosures: P.F. Hill: None. **D.X. Wang:** None. **M.D. Rugg:** None. **A.D. Ekstrom:** None. **D.R. King:** None. **B.C. Lega:** None.

Poster

P838. Hippocampal Interactions VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 2:45 PM - 3:45 PM

Program #/Poster #: P838.08

Topic: H.08. Learning and Memory

Support: DARPA RAM N66001-14-2-4032
NIH U01 NS113198

Title: Frequency-dependent response to intracranial theta-burst stimulation of anterior-temporal and posterior-medial divisions of the human hippocampal-cortical network

Authors: *M. S. HERMILLER[1], U. R. MOHAN[1], M. SPERLING[2], R. GORNIAK[2], A. SHARAN[2], G. A. WORRELL[3], B. M. BERRY[3], B. C. JOBST[4], K. DAVIS[5], R. E. GROSS[6], B. C. LEGA[7], S. DAS[5], J. STEIN[5], D. S. RIZZUTO[5], M. J. KAHANA[5], J. JACOBS[1]; [1]Columbia Univ., New York, NY; [2]Thomas Jefferson Univ. Hosp., Philadelphia, PA; [3]Mayo Clin., Rochester, MN; [4]Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [5]Univ. of Pennsylvania, Philadelphia, PA; [6]Emory Univ. Sch. Med., Atlanta, GA; [7]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Hippocampal interactions with a widespread set of brain regions, including the medial temporal, ventromedial prefrontal, lateral parietal, dorsal thalamus, precuneus and posterior medial cortex, give rise to a distributed hippocampal-cortical network (HCN). Theta-band activity is the putative neural mechanism that coordinates interactions between the hippocampus and the HCN, facilitates interregional communication, provides content for binding into memory, and supports recreation of content during retrieval. Proposed anatomical and functional subsystems of the HCN, the anterior-temporal (AT) and posterior-medial (PM) subnetworks, have been proposed to support specific memory processes. Anatomically, perirhinal and parahippocampal cortices show preferential connectivity with anterior and posterior hippocampus, respectively. Additionally, there is evidence of distinctions in theta-band activity in the human hippocampus, with low theta frequencies (~2-4 Hz) more prominent in the anterior hippocampus, and higher theta (~5-8 Hz) in the posterior hippocampus. Here, we tested the hypothesis that the AT and PM subnetworks would respond preferentially to brain stimulation delivered at different theta frequencies. We analyzed human electrocorticographic recordings from neurosurgical epilepsy patients (N=20) collected while systematically delivering direct electrical theta-burst stimulation varying in theta frequency (i.e., 3,4,5,6,7,8 Hz). We measured the amplitude and frequency of narrowband oscillations before and after stimulation events in recording electrodes placed in the AT (128 electrodes) and PM (50 electrodes) subnetworks. We found that electrodes in the AT exhibited power in lower theta-frequencies (~2-4 Hz) following stimulation relative to pre-stimulation, whereas electrodes in the PM showed an increase in higher theta (~5-8 Hz) power following stimulation. These results indicate that the AT and PM subdivisions of the human HCN respond differently to theta-burst stimulation, possibly related to the endogenous theta oscillations identified in the anterior versus posterior hippocampus. Our findings provide insight into how brain stimulation may be used to target endogenous network oscillations and motivate future interventions for therapies utilizing brain stimulation to treat disorders and impairments with an associated disruption in network oscillatory activity.

Disclosures: M.S. Hermiller: None. **U.R. Mohan:** None. **M. Sperling:** None. **R. Gorniak:** None. **A. Sharan:** None. **G.A. Worrell:** None. **B.M. Berry:** None. **B.C. Jobst:** None. **K. Davis:** None. **R.E. Gross:** None. **B.C. Lega:** None. **S. Das:** None. **J. Stein:** None. **D.S. Rizzuto:** A. Employment/Salary (full or part-time):; Nia Therapeutics. **M.J. Kahana:** A. Employment/Salary (full or part-time):; Nia Therapeutics. **J. Jacobs:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.01

Topic: A.07. Developmental Disorders

Support: TRDRP 28IP-0026

Title: Combined Prenatal Nicotine and THC Exposure via E-Cigarettes Alters Maternal Factors and Behavioral Development in Rats

Authors: K. R. BREIT[1], C. G. RODRIGUEZ[2], S. HUSSAIN[2], A. RUIZ[2], *J. THOMAS[2]; [1]Behavioral Teratology, West Chester Univ., West Chester, PA; [2]San Diego State Univ., San Diego, CA

Abstract: Nicotine and cannabis are two of the most commonly consumed drugs among pregnant women, often consumed via e-cigarettes. An estimated 5-14% of women report using e-cigarettes during their pregnancy, partly due to the assumption that e-cigarettes are safer than traditional smoking routes. However, the consequences of prenatal e-cigarette use with either drug, or the combination, are not well understood. This study exposed pregnant Sprague-Dawley rats to nicotine (36 mg/mL), tetrahydrocannabinol (THC; 100 mg/mL), the combination, or a vehicle (propylene glycol) via e-cigarettes daily from gestational days 5-20, mimicking the first and second human trimesters. Maternal blood samples were collected throughout the pregnancy at multiple time points to measure drug and metabolite levels, as were core body temperatures. Pregnant dams exposed to combined nicotine and THC had lower plasma nicotine and cotinine levels than those exposed to nicotine alone; similarly, the combined exposure group also had lower plasma THC and THC metabolite levels than those exposed to THC alone, illustrating pharmacokinetic interactions. Prenatal nicotine exposure gradually reduced basal core body temperatures during pregnancy, whereas exposure to THC alone decreased temperatures only during the drug exposure sessions. Despite these physiological effects, no changes were observed in food or water intake, weight gain, or basic litter outcomes. One sex pair of offspring per litter were used to separately examine motor development, activity levels, anxiety-related behaviors, and learning and memory. The combination of prenatal exposure to nicotine and THC delayed early sensorimotor development, and although nicotine and THC separately impaired later motor

coordination, the combination produced more severe motor deficits. In contrast, primarily unique effects of prenatal nicotine and THC were seen on other tasks. For example, prenatal exposure to nicotine reduced locomotor activity but increased fine motor activity in the open field, increased time spent in the open arms, and impaired visuospatial learning acquisition on the Morris water maze. In contrast, prenatal exposure to THC produced subtle and sex-dependent changes in both open field activity and anxiety-related behaviors (increased anxiety). These data suggest that combined exposure to prenatal exposure to low-moderate nicotine and THC elicits both unique and interactive effects on dams and offspring. These data can help identify risks and improve education for pregnant mothers about prenatal e-cigarette use. Supported by TRDRP 28IP-0026.

Disclosures: K.R. Breit: None. **C.G. Rodriguez:** None. **S. Hussain:** None. **A. Ruiz:** None. **J. Thomas:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.02

Topic: A.07. Developmental Disorders

Support: NIH Grant R00NS076661
NIH Grant R01NS097537
NIH Grant R01NS087031

Title: Select roles for ERK1/2 signaling in MGE-derived cells

Authors: *S. KNOWLES[1], J. S. MARTINEZ-FUENTES[1], K. REES[1], A. AFSHARI[1], M. HOLTER[1], K. NISHIMURA[1], G. LI[2], T. R. ANDERSON[2], J. NEWBERN[1];
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Abstract: The canonical RAS/RAF/MEK/ERK1/2 (ERK1/2) pathway is a highly conserved signaling cascade found in nearly all cells which is activated by diverse extracellular signals during development. Dysregulated ERK1/2 signaling is involved in multiple neurodevelopmental syndromes, particularly the RASopathies and select genetic forms of Autism Spectrum Disorder (ASD). It remains unclear which cell types are driving cognitive dysfunction in each of these disorders. The consequences of reduced ERK1/2 on the development and basic functions of MGE-derived GABAergic interneurons and glia are poorly understood. Here, we used a conditional genetic approach to specifically delete the principal ERK1/2 components *Mapk3/Erk1* (*Erk1*$^{-/-}$) and *Mapk1/Erk2* (*Erk2*$^{fl/fl}$) from MGE progenitors or GABAergic interneurons. Loss of ERK1/2 in progenitors definitively reduces glial number in the anterior commissure. We determined that ERK1/2 is not required for the establishment of cortical

GABAergic interneuron number or the expression of parvalbumin, a marker for a large subset of interneurons. However, our data reveal that ERK1/2 is necessary for the expression of SST, a marker of ~30% of cortical interneurons. We also examined the effects of a hyperactive *caMEK1* allele on SST neuron specification. Due to well established roles of ERK1/2 in activity-dependent development, we then tested whether the GABAergic neuron response to *in vivo* stimulation was altered by loss of ERK1/2. We found that ERK1/2 deleted GABAergic neurons exhibit deficits in FosB expression following chemogenetic stimulation. Interestingly, 1 week of chemogenetic stimulation led to a partial rescue of SST expression in ERK1/2 deleted neurons. Injection of a Cre-dependent AAV9-CAG-RFP into the neonatal somatosensory cortex was used to examine whether the development of dendritic complexity relies upon ERK1/2 signaling. Overall, our data highlight the important roles of ERK1/2 in the development and maturation of cortical GABAergic circuitry.

Disclosures: S. Knowles: None. **J.S. Martinez-Fuentes:** None. **K. Rees:** None. **A. Afshari:** None. **M. Holter:** None. **K. Nishimura:** None. **G. Li:** None. **T.R. Anderson:** None. **J. Newbern:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.03

Topic: A.07. Developmental Disorders

Title: Loss of p120-catenins leads to progressive hydrocephalus and cortical heterotopia in the developing mouse brain.

Authors: *J. RAKOTOMAMONJY[1], K. ZANG[2], Y.-G. NG[3], L. REICHARDT[4], A. CHENN[5], A. D. GUEMEZ-GAMBOA[6];
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Abstract: Members of the p120-catenin subfamily of catenins are crucial components of the cadherin-catenin cell adhesion complex as they stabilize cadherins at the cell surface. The p120-catenin subfamily consists of four members encoded by four independent genes. These four genes can be divided in 2 monophyletic groups: one containing ARVCF (*ARVCF*) and p120-catenin (*CTNND1*); the other comprising δ-catenin (*CTNND2*), and p0071 (*PKP4*). Previous work has shown that both p120-catenin and δ-catenin were crucial for proper dendritic spine, arborization, and synapse development. However, little is known about ARVCF and p0071 functions in the brain. Here, we investigated the role of evolutionarily related p120-catenin and

Arvcf in cortical development. To do so, we used stand-alone constitutive *Arcvf* and dorsal forebrain-specific *Ctnnd1* knockout (KO) mouse models, as well as a combination of both in order to assess their potential functional redundancy. Our results show that only the double KO mice exhibit reduced viability. Apicobasal polarity and cell junctions are disrupted in radial glia, resulting in formation of rosette-shaped heterotopias appearing as early as E16.5 and impaired postnatal cortical lamination. Moreover, p120/ARVCF double KO results in failed ependymal cell differentiation with loss of ciliated cells from the ventricular surface, potentially promoting development of hydrocephalus. Thus, our results suggest that p120-catenin and Arvcf have redundant functions during the neurogenic and gliogenic phases of cortical development.

Disclosures: J. Rakotomamonjy: None. **K. Zang:** None. **Y. Ng:** None. **L. Reichardt:** None. **A. Chenn:** None. **A.D. Guemez-Gamboa:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.04

Topic: A.07. Developmental Disorders

Support: MH117177-03

Title: Maternal Western-Style Diet Decreases Social Engagement in Japanese Macaques

Authors: *A. MITCHELL[1], S. KHAMBADKONE[3], G. DUNN[4], H. GUSTAFSSON[2], E. L. SULLIVAN[2];
[1]Behavioral Neurosci., [2]Psychiatry, Oregon Hlth. and Sci. Univ., Portland, OR; [3]Psychiatry and Behavioral Sci., John Hopkins Univ., Baltimore, MD; [4]Univ. of Oregon, Eugene, OR

Abstract: The CDC reports that almost 30% of women in the US were diagnosed with maternal obesity in 2019. Maternal obesity is associated with increased risk of offspring neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD). Although characterizations of the behavioral phenotypes underlying these disorders are becoming increasingly accurate, less is known about their neurodevelopmental origins. Increasing evidence from both epidemiological and preclinical animal models suggests that maternal overnutrition alters fetal neurodevelopment in ways that impair socio-emotional control in offspring. Impairments in socio-emotional control often result in social deficits, however the effects of mWSD on social development has not been examined in the macaque. The present study utilizes Japanese macaques to investigate the impact of maternal Western-Style Diet (WSD) (high in saturated fat and simple sugars) on offspring social behavior during novel peer introductions during the periweaning period. Our results suggest that maternal

WSD (mWSD) decreases social engagement in offspring, where mWSD offspring display decreases in the percentage of initiate proximity to the novel peer (β = -.37, p =.024). Significant increases in maternal IL-12 from exposure to the WSD (β =.33, p<.000) mediated decreases in initated affiliative contact observed in mWSD offspring (IL-2◊affiliative contact β= -.65, p < .000). We also found that maternal IL-12 were also associated with the decreased iniated proximity mWSD offspring (IL-12◊proximity β = -.35. p = .041). Interesting, alterations in offspring social behavior were also related to prepregnancy levels of leptin and glucose regulation (as assessed by glucose area under the curve). These results indicate that maternal exposure to an obesogenic diet prior to and during pregnancy influences fetal brain development in ways that lead to decreased social engagement.

Disclosures: A. Mitchell: None. **S. Khambadkone:** None. **G. Dunn:** None. **H. Gustafsson:** None. **E.L. Sullivan:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.05

Topic: A.07. Developmental Disorders

Support: NIH Grant GM103427

Title: Modeling CPT II Deficiency and Brain Function in Zebrafish

Authors: *C. BAKER[1], A. MARTA[2], N. ZIMMERMAN[2], R. WICKRAMASKARA[3], K. KRAMER[1], H. STESSMAN[3], E. HAAS[4], Z. KORADE[5], A. SHIBATA[2];
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Abstract: Mitochondrial oxidative phosphorylation supplies ATP to the brain except during early development and periods of starvation when neurons require ketone bodies produced by astrocytes or the liver for beta-oxidation and ATP synthesis. Beta-oxidation of long-chain fatty acids (LCFAs) depends upon the carnitine transferase system composed of carnitine palmitoyltransferase (CPT). CPT catalyzes the carnitine-dependent entry of long-chain acyl-Coenzyme A into the mitochondria matrix. Acylcarnitine production is important for membrane composition, protein synthesis and function, antioxidant production and cholinergic neurotransmission. Human CPTII deficiencies cause neurodevelopmental abnormalities, however, little is known about the mechanisms of brain-specific metabolic deficiencies. We present a zebrafish model system to investigate CPTII function in vertebrate brain and body development. Translation (TB) and splice-blocking (SB) morpholinos were used to knockdown

CPTII expression in wildtype (WT) TuAB zebrafish. Scrambled morpholino-injected (SC) and uninjected TuAB (WT) zebrafish served as controls. At 5days post-injection, morphological, lipid deposition and behavior experiments were performed. Standard length in TB and SB fish decreased by 128.2± 17.9μM and 316.7± 48.2μM compared to SC fish, respectively ($p \leq 0.01$, $n < 50$, ±SEM). Distance to rump in TB and SB fish decreased by 168.5 ± 24.6μM and 534.5 ± 80.1μM compared to SC fish, respectively ($p \leq 0.0001$, $n < 50$, ±SEM). Distance between eyes in SB fish decreased by 48.5± 8.4μM compared to SC fish, respectively ($p \leq 0.001$, $n < 50$, ±SEM). Standard length, distance to rump, distance between eyes were not significantly different for WT and SC injected fish ($p > 0.05$, $n > 50$). SB exhibited significantly higher percentages of fish with curved tails (38%), cardiac edema (20%), and abnormal yolk sacs (10%) than SC and WT fish. Lipid deposition was more concentrated in the liver, swim bladder, and yolk sac in TB and SB fish compared to WT and SC fish. LC-MS/MS analyses demonstrated in SB and TB fish a significant difference in lipids and acylcarnitine modifications. Behavior analyses showed that freezing duration, bursting movement, and duration of burst movement were significantly different in TB and SB fish compared to WT and SC fish ($p \leq 0.01$, $n < 70$). Immunohistochemistry and electrophysiological experiments will determine differences in network formation. This vertebrate model system serves to inform our understanding of the role of long chain fatty acid β-oxidation in neurodevelopment and how dysfunction of metabolic cell-signaling contributes to neurodevelopmental pathologies.

Disclosures: C. Baker: None. **A. Marta:** None. **N. Zimmerman:** None. **R. Wickramaskara:** None. **K. Kramer:** None. **H. Stessman:** None. **E. Haas:** None. **Z. Korade:** None. **A. Shibata:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.06

Topic: A.07. Developmental Disorders

Title: Investigation of a recurrent splice site mutation in mitochondrial methionyl tRNA formyl transferase (MTFMT) using cellular and a novel mouse model

Authors: *S. KANESHAMOORTHY[1,3], S. BELNAP[1], R. GUPTA[1], P. SWAMINATHAN[1], C. C4RCD RESEARCH GROUP[2], V. NARAYANAN[4], S. RANGASAMY[5];
[1]Tgen, Phoenix, AZ; [2]Tgen, Tempe, AZ; [3]Neurogenomics, TGen, Phoenix, AZ; [4]Ctr. for Rare Childhood Disorders, Translational Genomics Res. Inst., Phoenix, AZ; [5]Neurogenomics Div., Translational Genomics Res. Inst. (TGen), Phoenix, AZ

Abstract: Investigation of a recurrent splice site mutation in mitochondrial methionyl-tRNA formyl transferase (MTFMT) using cellular and a novel mouse model.Author: Sukanniya Kaneshamoorthy[1], Spencer Belnap[1,], Raj Gupta[1], Preethi Swaminathan[1], C4RCD Research Group[1, 2], Vinodh Narayanan[1,2], Sampathkumar Rangasamy[1,2] 1) Neurogenomics Division, Translational Genomics Research Institute (TGen), Phoenix, AZ2) Center for Rare Childhood Disorders (C4RCD), Translational Genomics Research Institute (TGen), Phoenix AZMutation in the MTFMT gene is identified to cause combined oxidative phosphorylation deficiency and Leigh syndrome. We recently identified a recurrent homozygous nonsynonymous mutation in *MTFMT* c. 626C>T, in three children from two unrelated families through whole-exome sequencing (WES) analysis. Although *MTFMT* mutations are established as a cause of LS and combined oxidative phosphorylation, the underlying molecular mechanisms of disease pathogenesis remain poorly understood. To understand the mitochondrial dysfunction in MTFMT mutation, we studied patient-derived fibroblast cells from homozygotes (probands) and six heterozygotes (carriers) from the two families. RNA sequence analyses revealed differentially expressed mitochondrial genes that separated affected patients and unaffected controls. We also found that glutaminase, a vital enzyme that helps in the conversion of glutamine to glutamate, is highly expressed in homozygous patients. This result indicates the activation of a compensatory metabolic pathway in homozygous patients due to the primary oxidative phosphorylation deficiency. MTFMT homozygote cells displayed elevated levels of mitochondrial reactive oxygen species (mROS) and decreased mitochondrial membrane potential, as measured by fluorimetry assay. Western blot analysis of OXPHOS complexes from the homozygous MTFMT mutant patient fibroblast cells revealed complex I and IV deficiency compared to heterozygotes and control cells. In collaboration with Jackson labs, we have created heterozygous MTFMT-Mut-Hum (mutant MTFMT with c. 626C>T variant human exon 4). Breeding of these heterozygous resulted in successful generation of homozygous MTFMT-Mut-Hum animals that recapitulate the mitochondrial protein synthesis defect. Our preliminary analysis signifies a consistent decrease in the level of nuclear-encoded CI subunit (NDUFB8) and mitochondrial encoded CIV subunit (MTCO1) in the mutant mice compared to the control mice. Our results demonstrate that loss of function MTFMT (c. 626C>T) mutation leads to altered mitochondrial homeostasis and energy metabolism.

Disclosures: S. kaneshamoorthy: None. **S. Belnap:** None. **R. Gupta:** None. **P. Swaminathan:** None. **C. C4RCD Research Group:** None. **V. Narayanan:** None. **S. Rangasamy:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.07

Topic: A.07. Developmental Disorders

Support: SNF Grant 31003A-175778

Title: The Slc6a8$^{y/Y389C}$ rat model of creatine transporter deficiency reveals behavioral disorder, altered brain metabolism, muscle atrophy and altered motor function

Authors: *L. DURAN-TRIO[1], G. FERNANDES-PIRES[1], D. SIMICIC[2], J. GROSSE[2], C. ROUX-PETRONELLI[1], S. BRUCE[1], P.-A. BINZ[1], C. SANDI[2], C. CUDALBU[2], O. BRAISSANT[1];
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Abstract: Creatine is an organic compound used as fast phosphate energy buffer to recycle ATP, important in tissues with high energy demand such as muscle or brain. Creatine is taken from the diet or endogenously synthetized by the enzymes AGAT and GAMT, and specifically taken up by the transporter SLC6A8. Deficit in the endogenous synthesis or in the transport leads to Cerebral Creatine Deficiency Syndromes (CCDS). CCDS are characterized by brain creatine deficiency, intellectual disability with severe speech delay, behavioral troubles such as attention deficits and/or autistic features, epilepsy and motor dysfunction. Among CCDS, the X-linked creatine transporter deficiency (CTD) is the most prevalent with no efficient treatment so far. Different mouse models of CTD were generated by doing long deletions in the *Slc6a8* gene showing reduced brain creatine and cognitive deficiencies or impaired motor function. Our recently described *Slc6a8$^{y/Y389C}$* knock-in (KI) rat model of CTD holds an identical point mutation found in patients with reported lack of transporter activity. KI males showed brain creatine deficiency, increased urinary creatine/creatinine ratio, cognitive deficits, autistic-like traits, motor dysfunction and muscle atrophy. *Slc6a8^{Y389C}* KI rat fairly enriches the spectrum of CTD models and provides new data about the pathology, being the first animal model of CTD carrying a point mutation.

Disclosures: L. Duran-Trio: None. **G. Fernandes-Pires:** None. **D. Simicic:** None. **J. Grosse:** None. **C. Roux-Petronelli:** None. **S. Bruce:** None. **P. Binz:** None. **C. Sandi:** None. **C. Cudalbu:** None. **O. Braissant:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.08

Topic: A.07. Developmental Disorders

Support: NIH Grant NS110586

Title: Effect of Post-Infectious Hydrocephalus on the Developing Ventricular-Subventricular Zone Stem Cell Niche

Authors: *J. HERMAN[1], M. MARTLAND[1], S. KALARIA[1], Y. WANG[1], F. MANDINO[2], A. MEZGER[1], E. M. LAKE[2], P. H. VERARDI[1], J. C. CONOVER[3];
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Abstract: Congenital hydrocephalus, a common birth defect affecting 1 in 1000 infants, is characterized by expansion of the cerebroventricular system. One common cause of this disorder is infection, resulting in post-infectious hydrocephalus (PIH). PIH can occur following maternal infection during pregnancy, resulting in viral and inflammatory exposure to the developing fetal and neonatal brain. Our group is interested in the effects of PIH on the ventricular-subventricular zone (V-SVZ), which includes an active stem cell niche present throughout development and then retained only along the lateral wall of the lateral ventricles postnatally. V-SVZ stem cells populate the developing brain with newly generated neurons and glial cells, including multi-ciliated ependymal cells which are generated through the process of ependymogenesis. A monolayer of ependymal cells then lines the ventricles providing a protective barrier and fluid/solute exchange system, and, via motile cilia, laminar flow of cerebrospinal fluid at the ventricle surface. Here, we present a novel mouse model of PIH using intracerebroventricular injections of influenza or a non-virulent component of influenza (neuraminidase) at two specific timepoints: embryonic day 16, prior to the development of the ependyma, and neonatal at postnatal day 4, following completion of the ependymal monolayer. Influenza and neuraminidase alone were found by immunohistochemistry to target ependymal cells and induce PIH in approximately 60% of mice injected postnatally as observed by serial section analysis of the lateral ventricles or by 11.7T MRI. Ependymal denudation and accompanying astrogliosis were noted along the expanded ventricle surface based on immunohistochemical analysis. This model will be used to assess inflammatory regulation in the development of PIH and V-SVZ repair mechanisms, as well as the niche's capacity for continued neurogenesis over time.

Disclosures: J. Herman: None. **M. Martland:** None. **S. Kalaria:** None. **Y. Wang:** None. **F. Mandino:** None. **A. Mezger:** None. **E.M. Lake:** None. **P.H. Verardi:** None. **J.C. Conover:** None.

Poster

P062. Developmental Disorders: Animal Models of Developmental Disorders Other Than Autism II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P062.09

Topic: A.07. Developmental Disorders

Title: Evaluating the impact of gestational ethanol consumption on adult immune function and addiction-like behavior.

Authors: *P. KOZAN, B. O. GLEESON, K. L. RONALD, P. D. RIVERA;
Dept. of Biol., Hope Col., Holland, MI

Abstract: In the United States, one in twenty babies are born with fetal alcohol spectrum disorder (FASD). FASD is commonly associated with maternal alcohol consumption during pregnancy that results in offspring with physiological and behavioral deficits that are mediated by immune dysfunction. Previous studies in rodents have found that gestational ethanol exposure leads to increased inflammatory responses and deficits in cognitive and social behaviors. Additionally, enhanced immune signaling can lead to an increase in addiction-like behavior in rodents. However, studies have not characterized the impact of gestational alcohol on adult addiction-like behaviors, as it relates to systemic immune dysfunction. Therefore, to assess the impact of gestational ethanol on adult immune function and subsequent addiction-like behavior, we used WT C57BL/6J dams (PND50) and bred them with WT C57Bl/6J males. Dams were then checked for plugs daily and drinking-in-the-dark (DID) occurred at gestation day (GD) 10-14. At PND 4 and 10, pups were removed from their litter to record ultrasonic vocalizations (USVs). Preliminary results suggest a sex by treatment effect in the number of USVs emitted from pups (PND4). Offspring from dams that previously consumed EtOH during GD 10-14, appear to vocalize more than water controls. Offspring matured to adulthood and addiction-like behavior was assessed via DID. Mice (PND60-70) were sacrificed 90 minutes after DID test day. Plasma and brain samples were then collected to examine systemic inflammatory cytokines in plasma via ELISAs and microglia and Fos (Iba1+) via IHC, respectively. Taken together, we hope to understand the impact of gestational EtOH exposure on physiological development, adult immune function, and addiction-like behaviors. Future experiments will replicate this process using other drugs of abuse, such as morphine.

Disclosures: P. Kozan: None. **B.O. Gleeson:** None. **K.L. Ronald:** None. **P.D. Rivera:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.01

Topic: A.03. Stem Cells and Reprogramming

Support: National Institute of Mental Health Grant MH109648-05, MH115716-04
Simons Foundation Awards 399558; 632742)

Title: Autism spectrum disorder heterogeneity modeled in iPSC-derived telencephalic organoids

Authors: *A. JOURDON[1], J. MARIANI[1], F. WU[1], D. CAPAUTO[1], S. NORTON[1], A. AMIRI[1], J. SCHREINER[1], L. TOMASINI[1], A. ABYZOV[3], F. M. VACCARINO[1,2];
[1]Child Study Ctr., [2]Neurosci., Yale Univ., New Haven, CT; [3]HSR, Harwick 3-12, Mayo Clin., Rochester, MN

Abstract: Autism spectrum disorder (ASD) exhibits heterogeneity in clinical presentation and genetic studies have not yet identified a convergent etiology for the spectrum. Using induced pluripotent stem cell (iPSC)-derived brain organoids, we modeled in this study the alterations in the formation of the telencephalon between 18 idiopathic ASD individuals and their unaffected first-degree family members as controls. Differences in cellular composition and cell type-specific gene expression were evaluated by single-cell RNA-sequencing (scRNA-seq) during the first 3 months of organoid neural differentiation over a longitudinal time course. More than a hundred scRNA-seq samples were collected and integrated in the study presented here. This large-scale dataset allowed us to investigate the sources of variation influencing human neurogenesis in the organoid system over multiple genetic backgrounds and culture batches. Using correlation analysis, we showed how different patterns of gene expression in progenitor cells, such as canonical signaling pathway activity in radial glia, were associated with the propensity to generate different subtypes of excitatory and inhibitory lineages of the telencephalon. We observed that on average organoids generated from ASD probands did not present a specific cell type composition compared to control individuals, although differential gene expression revealed cell type-specific alterations in genes associated with neural patterning, cell division, cortical neurogenesis and synaptogenesis. We investigated the biological effect of macrocephaly (larger head circumference) on ASD probands gene expression, and propose head circumference as a clinical variable susceptible to drive the heterogeneity of ASD phenotypes observed in organoids. This suggests that macrocephaly could be a potential stratifying factor in large clinical or genetic studies of ASD. Altogether, the research presented here aims to understand the heterogeneity of human brain development across individuals and establish how large single-cell omics study on patient-derived organoids could decipher the etiologies behind neurodevelopmental disorders.

Disclosures: A. Jourdon: None. **J. Mariani:** None. **F. Wu:** None. **D. Capauto:** None. **S. Norton:** None. **A. Amiri:** None. **J. Schreiner:** None. **L. Tomasini:** None. **A. Abyzov:** None. **F.M. Vaccarino:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH (NINDS) Grant U54NS117170 (JMP)
NSF Graduate Research Fellowship Program (MCV)

Title: Human induced pluripotent stem cell-derived neuronal and brain organoid models of SLC6A1-related neurodevelopmental disorder

Authors: *M. C. VARELA, T. N. THENSTEDT, J. G. VALDIVIESO, M. D. UHLER, J. M. PARENT;
The Univ. of Michigan, Ann Arbor, MI

Abstract: Epileptic encephalopathies and other neurodevelopmental disorders are linked to variants in the *SLC6A1* gene which encodes for the most abundant GABA transporter in the brain, GAT-1. GAT-1 is responsible for the reuptake of GABA at the synapse and, in the cortex, is expressed primarily in inhibitory neurons. Loss of GAT-1 function results in epilepsy and cognitive delays, however how GAT-1 haploinsufficiency affects early cortical development in humans remains unclear. In this study, we use both 2D and 3D human induced pluripotent stem cell (iPSC)-derived models to test the hypothesis that GAT-1 LOF alters inhibitory neuron development and subsequent network formation. We first reprogrammed human foreskin fibroblasts into iPSCs with concurrent CRISPR/Cas9 gene editing targeted to generate out-of-frame insertions or deletions in the *SLC6A1* gene. We generated several *SLC6A1* heterozygous (Het) null, compound heterozygous (KO), and isogenic control (WT) iPSC lines which were differentiated into 3D cortical and ventral organoids using our novel self-organizing single rosette spheroid (SOSRS) model. We also generated 2D induced GABAergic neurons (iGNs) using an inducible expression system driving the human ASCL1 and DLX2 transcription factors. We used a combination of immunocytochemistry and RT-qPCR of SOSRS cultured for 2-36 weeks or iGNs for 1-4 weeks to investigate interneuron development and specification. Because loss-of-function *SLC6A1* variants are predicted to increase ambient GABA levels in early brain development, and ambient GABA levels influence the migration of developing interneurons, we also fused cortical and ventral SOSRS to investigate interneuron migration and integration into the cortex. Both our 2D and 3D models displayed the expected GABAergic and mature neuronal marker expression and morphology. We found a marked reduction of GAT-1 expression in the KO iGNs and SOSRS ranging from 6.7 ± 2.0% to 28.2 ± 11.5% of WT, while GAT-1 expression in the Het lines was partially reduced, ranging from 23.4 ± 16.5% to 56.4 ± 33.4% of WT (n=3 lines each). We observed a potential delayed migration phenotype in mutant *SLC6A1* ventral SOSRS compared to controls, with the KO SOSRS displaying severely decreased neuronal migration, and Hets showing an intermediate degree of decreased migration. Our findings suggest that *SLC6A1* complete loss of function, and to a lesser extent haploinsufficiency, impairs GABAergic cell migration and likely alters subsequent network formation. Further investigation into this phenotype could provide early treatment targets for *SLC6A1*-related neurodevelopmental disorders.

Disclosures: M.C. Varela: None. **T.N. Thenstedt:** None. **J.G. Valdivieso:** None. **M.D. Uhler:** None. **J.M. Parent:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.03

Topic: A.03. Stem Cells and Reprogramming

Support: NIMH Grant MH109648-05
NIMH Grant MH115716-04
Simons Foundation Award 399558
Simons Foundation Award 632742

Title: Parallel RNA-seq and ChIP-seq in iPSC-derived cortical organoids identify important regulators of neuronal development in ASD

Authors: *S. NORTON[1], J. MARIANI[1], A. JOURDON[1], D. CAPAUTO[1], L. TOMASINI[1], J. SCHREINER[1], A. PANDA[2], Y. WANG[2], F. WU[1,3], A. ABYZOV[2], F. VACCARINO[1]; [1]Yale Univ., New Haven, CT; [2]Mayo Clin., Rochester, MN; [3]Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: Autism spectrum disorders (ASD) exhibit high variation between patients, with more severe disorder correlated with larger head size (macrocephaly) relative to the general population (normocephaly). Cortical organoids derived from induced pluripotent stem cells (iPSC) provide an effective model of early brain development (Mariani 2015, Amiri 2018) and allow for the retrospective study of neurodevelopment in individuals with ASD. We derived iPSC lines from six nine macrocephalic and five normocephalic ASD probands along with their respective, unaffected fathers (total, XX14 subjects). We differentiated these lines into cortical organoids and analyzed gene expression over a developmental timeline from neural stem cells to cortical neurons by RNA sequencing. In parallel we mapped gene regulatory elements (enhancers) location and activity by histone modifications (ChipIP-seq) and open chromatin (ATAC-seq) at various stages of development. Putative enhancer elements defined from ChIP-seq analyses against four histone marks were linked to physically interacting gene promoters to identify likely cis-activators and repressors of gene expression. Such elements can harbor DNA sequence mutations that may impact their activity. Genes that significantly covary with head size and ASD diagnosis are expressed in excitatory neuron (EN) and inhibitory neuron (IN) lineages, indicating divergent lineage trajectories in macrocephalic vs normocephalic ASD. We highlight a set of enhancer elements that are likely regulators of genes for EN and IN lineages implicated in ASD, including NEUROD2, NEUROD6, DLX5, and FOXG1. Our strategy allows to construct a gene regulatory network of early brain development in typical development and developmental disorders such as ASD.

Disclosures: S. Norton: None. **J. Mariani:** None. **A. Jourdon:** None. **D. Capauto:** None. **L. Tomasini:** None. **J. Schreiner:** None. **A. Panda:** None. **Y. Wang:** None. **F. Wu:** None. **A. Abyzov:** None. **F. Vaccarino:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.04

Topic: A.03. Stem Cells and Reprogramming

Support: CrestOptics-IIT JointLab for Advanced Microscopy
Regione Lazio MARBEL Life2020
Regione Lazio Bio3DBrain FSE 2014-2020
Sapienza University

Title: Modelling FragileX syndrome using iPSCS derived cerebral organoids

Authors: C. BRIGHI[1,3], A. SOLOPERTO[1], F. SALARIS[1], M. ROSITO[1], F. CORDELLA[1,3], S. GHIRGA[1], V. DE TURRIS[1], P. F. PORCEDDU[2], C. D'ANTONI[1], A. REGGIANI[2], A. ROSA[1,4], ***S. DI ANGELANTONIO**[1,3];
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Abstract: Fragile X syndrome (FXS) is a neurodevelopmental disorder, characterized by intellectual disability and sensory deficits, caused by epigenetic silencing of the *FMR1* gene and subsequent loss of its protein product, fragile X mental retardation protein (FMRP). Delays in synaptic and neuronal development in the cortex have been reported in FXS mouse models, however, the main goal of translating lab research into pharmacological treatments in clinical trials has been so far largely unsuccessful, leaving FXS a still incurable disease. Here, we generated 2D and 3D in vitro human FXS model systems based on isogenic *FMR1* knock-out mutant and wild-type human induced pluripotent stem cell (hiPSC) lines. Phenotypical and functional characterization of cortical neurons derived from FMRP-deficient hiPSCs display altered gene expression and impaired differentiation when compared with the healthy counterpart. FXS cortical cultures show increased proliferation of GFAP positive cells, likely astrocytes, increased spontaneous network activity and depolarizing GABAergic transmission. Cortical brain organoid models show increased proliferation of glial cells, and bigger organoid size. Our findings demonstrate that FMRP is required to correctly support neuronal and glial cell proliferation, and to set the correct excitation/inhibition ratio in human brain development.

Disclosures: C. Brighi: None. **A. Soloperto:** None. **F. Salaris:** None. **M. Rosito:** None. **F. Cordella:** None. **S. Ghirga:** None. **V. de Turris:** None. **P.F. Porceddu:** None. **C. D'Antoni:** None. **A. Reggiani:** None. **A. Rosa:** None. **S. Di angelantonio:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.05

Topic: A.03. Stem Cells and Reprogramming

Support: Exploration Grant NFRFE201900425 to J.G.M.
CFI Grant 037755 to J.L.
2020 Scottish Rite Charitable Foundation of Canada Grant to J.L.

Title: Proteomics of Δ^9-tetrahydrocannabinol (THC) impact on cortical development in health and schizophrenia with iPSC-derived cerebral organoids

Authors: B. ALURAL[1], B. BALL[1], J. ANG[1], H. THORPE[2], S. D. SHERIDAN[3], R. H. PERLIS[3], J. KHOKHAR[2], J. GEDDES-MCALISTER[1], *J. LALONDE[1];
[1]Mol. and Cell. Biol., [2]Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada; [3]Ctr. for Quantitative Health, Ctr. for Genomic Med. and Dept. of Psychiatry, Massachusetts Gen. Hosp., Boston, MA

Abstract: The primary psychoactive compound of cannabis, Δ^9-tetrahydrocannabinol (THC), causes depression of glutamate signaling and functional changes in the brain as a result of its interaction with cannabinoid receptor type 1 (CB_1) receptors. Neuroimaging studies have also identified structural changes in regular cannabis users. Given these findings, an important question concerns the impact that THC exposure may have on the growth and organization of neuronal cells during the prenatal stages of brain development. Study of cerebral cortex formation at the cellular and molecular levels has been largely limited to non-human models in the past; however, the possibility of using human-induced pluripotent stem cells (hiPSCs) that can be differentiated as 3-dimensional organoids now offers the opportunity to investigate baseline and dynamic changes in a human-like neural system. Here, we used cerebral organoid models derived from different hiPSC lines in conjunction with proteomics and imaging methods to profile the impact of THC exposure. Since previous studies established that cannabis-use rates and diagnosis of cannabis use disorder are more frequent in schizophrenia (SCZ) patients than non-patient groups, and that a connection between cannabis and first-episode psychosis exists for a subgroup of vulnerable patients, we also searched for possible differences specific to this neuropsychiatric disorder. First, we present characterization of the 6 hiPSC lines (3 SCZ patients and 3 healthy control individuals) used in our study, including their culture as cerebral organoids

alongside the expression of CB receptors (CB_1 and CB_2) and other common markers. Second, we report the completed quantitative proteomic analysis of our 6 hiPSCs lines matured for 125 days as organoids where we identified 37 proteins with significant difference in abundance between healthy control and SCZ specimens or resulting from THC application to the maturation culture media. Finally, we highlight ongoing efforts at understanding the connection between specific proteins that we found to be affected by THC, in particular those that are predominantly expressed in synaptic terminals. Together, our efforts provide tantalizing insights about the impact of THC on neuron biology during prenatal stages of cerebral cortex development, as well as possible connections to psychosis.

Disclosures: B. Alural: None. **B. Ball:** None. **J. Ang:** None. **H. Thorpe:** None. **S.D. Sheridan:** None. **R.H. Perlis:** None. **J. Khokhar:** None. **J. Geddes-McAlister:** None. **J. Lalonde:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.06

Topic: A.03. Stem Cells and Reprogramming

Support: FAPEMIG Grant APQ-00059-21
FAPEMIG Grant APQ-03744-17
CNPq Grant 441719/2020-1

Title: Schizophrenic individuals hiPSC-derived astrocytes impair synaptic engulfment by induced microglial-like cells (iMGs) in vitro

Authors: *P. LEAL CARDOZO[1], P. TRINDADE[3,4], J. P. S. LACERDA[1], J. S. FAHEL[1], R. C. CORDEIRO[5], J. ALVES-SILVA[2], L. CHICAYBAM[6], M. H. BONAMINO[6], S. K. REHEN[4,3], F. M. RIBEIRO[1];
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Abstract: Schizophrenia (SCZ) is a neuropsychiatric disorder caused by the interaction between genetic and environmental factors. SCZ individuals exhibit cognitive deficits, positive (psychosis, hallucinations and delusions) and negative symptoms (depression, avolition and anhedonia), as well as reduced gray matter volume. Microanatomical analyses suggest this phenomenon occurs due to diminished dendritic spine density, which likely happens as a result of exaggerated synaptic pruning. It has already been shown that the classical complement cascade and CX3CL1/CX3CR1 pathway play a direct role in this process, triggering synaptic

engulfment by microglia. Taking into account that pre-natal infection acts as an environmental risk factor for SCZ, this work aims at analyzing synaptic pruning-related modulators (complement components and CX3CL1) production employing induced pluripotent stem cells (iPSCs)-derived astrocytes from both healthy control (HC) and SCZ individuals (n = 5) after stimulation with TNF-α. All experiments were conducted in accordance with the Helsinki Declaration and approved by an IRB. The present results indicate that CX3CL1 transcripts are increased in SCZ-derived astrocytes relative to HC-derived astrocytes, while the classical complement components (C1q, C3 and C4) display similar expression levels across both phenotypes. Further validation indicates that secreted CX3CL1 levels mirror its transcript expression pattern, thus confirming the increased CX3CL1 production in SCZ astrocytes upon TNF-α stimulation. In order to verify whether SCZ astrocytic secreted CX3CL1 stimulates higher synaptic engulfment by microglia, HC induced microglial-like cells (iMGs) were incubated with astrocyte conditioned media (ACM) from both phenotypes, under non-stimulated (N.S.) or TNF-α stimulated conditions and in the presence or absence of the CX3CR1 antagonist (AZD 8797) for up to 24 h with CM-Dil-labeled synaptoneurosomes. Surprisingly, TNF-α HC ACM, N.S. SCZ ACM and TNF-α HC ACM exposure lead to lower synaptoneurosomal phagocytic engulfment by iMGs relative to N.S. HC ACM. In addition, pre-treatment with AZD 8797 enhances synaptic phagocytosis only in iMGs exposed to N.S. HC ACM, with no changes observed in other conditions. Since secreted cytokines can impact microglial phagocytic capacity, we analyzed cytokine expression in HC and SCZ astrocytes stimulated with TNF-α. Interestingly, TNF-α and IL-6 mRNA levels are elevated in stimulated SCZ astrocytes relative to HC astrocytes. Altogether, these results indicate that SCZ astrocytes secreted factors impair HC iMG synaptic engulfment.

Disclosures: P. Leal Cardozo: None. **P. Trindade:** None. **J.P.S. Lacerda:** None. **J.S. Fahel:** None. **R.C. Cordeiro:** None. **J. Alves-Silva:** None. **L. Chicaybam:** None. **M.H. Bonamino:** None. **S.K. Rehen:** None. **F.M. Ribeiro:** None.

Poster

P067. Human Stem Cell and Organoid Models in Autism and Neurodevelopmental Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P067.07

Topic: A.03. Stem Cells and Reprogramming

Support: ERA-NET NEURON-102 SYNSCHIZ grant (NWO) 013-17-003 4538
ERA-NET NEURON DECODE! grant (NWO) 013.18.001
NWO-CAS grant 012.200.001
the Netherlands Organization for Health Research and Development ZonMw grant 91217055

SFARI grant 610264
South-Eastern Norway Regional Health Authority #2018094
China scholarship council 201806210076

Title: Loss-of-function variants in the schizophrenia risk gene SETD1A alter neuronal network activity in human neurons through cAMP/PKA pathway

Authors: S. WANG[1], J.-R. VAN RHIJN[1], I. AKKOUH[2], N. KOGO[1], N. MAAS[1], A. BLEECK[1], E. LEWERISSA[1], K. WU[1], C. SCHOENMAKER[1], S. DJUROVIC[2], H. VAN BOKHOVEN[1], T. KLEEFSTRA[1], N. NADIF KASRI[1], D. SCHUBERT[1];
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Abstract: <META NAME="author" CONTENT="王 珊">**Abstract** Heterozygous loss-of-function (LoF) mutations in *SETD1A*, which encodes a subunit of histone H3 lysine 4 methyltransferase, have been shown to cause a novel neurodevelopmental syndrome and increase the risk for schizophrenia. To study the effect of decreased SETD1A function in human cells, we generated excitatory/inhibitory neuronal networks from human induced pluripotent stem cells with a *SETD1A* heterozygous LoF mutation (*SETD1A*$^{+/-}$). Our data show that *SETD1A* haploinsufficiency resulted in altered neuronal network activity, which was mainly characterized by an overly synchronized network. In individual neurons, this network phenotype was reflected by increased somatodendritic complexity and elevated synaptic connectivity. We found that this network phenotype was strongly driven by *SETD1A* haploinsufficiency in glutamatergic neurons. In accordance with the functional changes, transcriptomic profiling revealed perturbations in gene sets associated with schizophrenia, synaptic transmission and glutamatergic synaptic function. At the molecular level, we identified specific changes in the cAMP/PKA pathway pointing toward a hyperactive cAMP pathway in *SETD1A*$^{+/-}$ neurons. Finally, using pharmacological experiments targeting the cAMP pathway we were able to rescue the network deficits in *SETD1A*$^{+/-}$ cultures. In conclusion, our results illuminate key molecular, cellular and network abnormalities resulting from *SETD1A* haploinsufficiency and demonstrate a potential link between SETD1A and the cAMP-dependent pathway in human neurons.

Disclosures: S. Wang: None. **J. van Rhijn:** None. **I. Akkouh:** None. **N. Kogo:** None. **N. Maas:** None. **A. Bleeck:** None. **E. Lewerissa:** None. **K. Wu:** None. **C. Schoenmaker:** None. **S. Djurovic:** None. **H. van Bokhoven:** None. **T. Kleefstra:** None. **N. Nadif Kasri:** None. **D. Schubert:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.01

Topic: B.07. Synaptic Plasticity

Support: NS045876
DC01919
T35HL007649
James G. Hirsch, MD Endowed Medical Student Research Fellowship
Jack and Francine Levin Fellowship

Title: Presynaptic plasticity in the calyx of Held synapse involves structural changes in F-actin and vesicle pools.

Authors: *S. SUBRAMANIAN[1], K. SAN AGUSTIN RUIZ[2], J. BURTON[1], M. E. GOODRIDGE[1], J. SONG[3], D. GOMEZ[1], L. K. KACZMAREK[1], E. A. JONAS[1];
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Abstract: Post-tetanic potentiation (PTP) is a form of short-term synaptic plasticity that occurs in part due to an increase in the number of readily releasable vesicles at the active zone. Clustering of the active zone structure is inversely correlated with the presence of filamentous actin (F-actin). The calyx of Held is a large high-fidelity synapse located in the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem. The calyx of Held contains a dense layer of presynaptic F-actin that is organized by the potassium channel Kv3.3 and facilitates endocytosis of presynaptic vesicles. We hypothesized that tetanic stimulation would result in reorganization of the F-actin structure in the presynaptic terminal, allowing the accumulation of additional vesicles at active zones and thus PTP. Initial confocal microscopy studies of rat brain slices containing the MNTB showed that in seconds after stimulation with potassium chloride, F-actin intensity near active zones decreased, while active zone size increased. Here, we report the use of three-dimensional stimulated emission depletion (3D STED) super resolution microscopy to reconstruct the presynaptic terminal before and after electrical stimulation of calyx of Held synapses. We found that F-actin likely depolymerizes 20-40s seconds after stimulation, concomitant with the expansion of docked vesicle pools. After 20 more seconds, the F-actin likely repolymerizes even as the number of docked vesicles continues to increase, suggesting that actin contributes to PTP through two separate mechanisms: (1) depolymerization in order to make space for active zone machinery and perhaps for recycling/reserve pool activity and (2) repolymerization, perhaps to coordinate endocytosis and the replenishment of vesicle pools. Since tethering by F-actin is known to coordinate complexes of vesicles and mitochondria in the calyx of Held, we plan to use electron microscopy to determine if there are changes in mitochondrial size, structure, or proximity to docked vesicles following tetanic stimulation. This study uses cutting-edge microscopy techniques to capture dynamic, activity-induced structural changes in the calyx of Held synapse that contribute to its rapid and sustained synaptic transmission.

Disclosures: S. Subramanian: None. **K. San Agustin Ruiz:** None. **J. Burton:** None. **M.E. Goodridge:** None. **J. Song:** None. **D. Gomez:** None. **L.K. Kaczmarek:** None. **E.A. Jonas:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.02

Topic: B.07. Synaptic Plasticity

Support: NIDA IRP

Title: Identifying markers of synaptic activation using flow cytometry in synaptoneurosome preparations

Authors: *F. RUBIO[1], D. E. OLIVARES[2], C. DUNN[5], S. ZHANG[2], P. V. SELVAM[6], E. M. HILAIRE[7], A. HENRY[6], C. J. NOGUERAS-ORTIZ[5], K. PEREZ DE ARCE[8], F. C. CRUZ[9], S. ZHANG[2], C. MEJIAS-APONTE[3], B. T. HOPE[4];
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Abstract: Neuroadaptations within glutamatergic terminals from the medial prefrontal cortex (mPFC) that synapse onto dendritic spines of medium spiny neurons in nucleus accumbens (NAc) are thought to be important for learning in animal models of drug addiction. Many studies used western blot assays to assess molecular alterations in synapses that were obtained from whole brain areas regardless of the afferent source of these synapses. We developed an optimized flow cytometry of synaptoneurosomes (FCS) protocol for analyzing protein and phosphorylation alterations in individual synaptoneurosomes from identified afferent sources – synaptoneurosomes contain sealed presynaptic and postsynaptic elements of a synapse. First, YFP-expressing AAV was injected into the PFC and the expressed YFP protein was transported over 6-8 weeks to label PFC-specific presynaptic endings in the nucleus accumbens core (NAcc). On test day, we injected each rat with 20 mg/kg cocaine (IP) and placed them in a novel environment to induce cellular and synaptic activation. We dissected the NAcc at 0, 5, 10, 30 and 60 min following the injection. We prepared synaptoneurosomes and confirmed their size and structure using electron microscopy and nanoparticle tracking analysis. We labeled the synaptoneurosomes with commercially available antibodies against candidate synaptic activation markers, such as Arc, CaMKII (protein and phosphorylation-specific), ribosomal protein S6 (protein and phosphorylation-specific) and calcineurin (protein and phosphorylation-specific). We then used our FCS protocol to quantify these molecular elements selectively in PFC-NAc synaptoneurosomes labeled with YFP. We found increased ribosomal S6 protein (but not phosphorylation) at 5-60 min and increased calcineurin at 5 and 10 min. We did not observe significant alterations in the Arc, CaMKII protein or phosphorylation at any of these time points. We validated the increases of ribosomal protein S6 and Calcineurin proteins in NAc synapses

using immunoelectron microscopy. Chloral hydrate anesthesia before cocaine injections will be used to demonstrate neuronal activity-dependence of cocaine-induced synaptic increases in S6 and calcineurin proteins. We propose that S6 and Calcineurin can be markers of synaptic activation *in vivo*. Our FCS-based approach is a rapid technique for discovery of synaptic plasticity alterations in small amounts of tissue obtained from single rats.

Disclosures: F. Rubio: None. **D.E. Olivares:** None. **C. Dunn:** None. **S. Zhang:** None. **P.V. Selvam:** None. **E.M. Hilaire:** None. **A. Henry:** None. **C.J. Nogueras-Ortiz:** None. **K. Perez De Arce:** None. **F.C. Cruz:** None. **S. Zhang:** None. **C. Mejias-Aponte:** None. **B.T. Hope:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.03

Topic: B.07. Synaptic Plasticity

Support: K99AG065645
R01AG042178
R01AG47812
R01NS105473
AG060767
AG069333
AG066347

Title: Synaptosomal vs cytosolic microRNAs and synapse function in Alzheimer's disease

Authors: *E. ORLOV[1], P. GOWDA[2], P. H. REDDY[3], S. KUMAR[4];
[1]Texas Tech. Univ., Luboock, TX; [2]Texas Tech. Univ. Hlth. Sci. Ctr., Luboock, TX; [3]Dept. of Intrnl. Med., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [4]TTUHSC, Lubbock, TX

Abstract: Synaptosomal vs cytosolic microRNAs and synapse function in Alzheimer's disease Erika Orlov[1], Prashanth Gowda[1], P. Hemachandra Reddy[1-5], Subodh Kumar[1],1. Internal Medicine Department, 2. Neuroscience & Pharmacology, 3. Neurology, 4. Public Health Department, 5. Speech, Language and Hearing Sciences, School Health Professions, Texas Tech University Health Sciences Center, Lubbock, TX, 79430 USA.
Introduction: The purpose of our study is to understand the role of synaptosomal microRNAs (miRNAs) in Alzheimer's disease (AD) progression. MiRNAs have been discovered in nerve terminals, synaptosomes, and synaptic vesicles in recent research. However, in AD, it is unclear if any specific miRNA is localized at the synapse. If so, are synapse miRNAs expressed differentially in the cytosol? What function do synaptosomal miRNAs play in synaptic activity

and neurotransmission in AD? **Methods:** To address these intriguing questions, synaptosomal and cytosolic fractions were separated from AD and healthy control postmortem brains. Synaptosomal fractions were confirmed by transmission electron microscopy, qRT-PCR and immunoblotting of synaptic proteins. Next, we performed the global affymetrix microarray analysis of synaptosomal and cytosolic miRNAs in both AD patients and healthy controls. Transcriptome Analysis Console V.4 was used to compare miRNAs data in four different ways: 1) AD synaptosome vs AD cytosol, 2) healthy control (HC) synaptosome vs HC cytosol, 3) AD cytosol vs HC cytosol, and 4) AD synaptosome vs HC synaptosome. **Results:** In different comparisons, some miRNAs tended to display very significant ($P<0.0001$) and high fold changes variance (+/- 200-fold) in their expression levels. Validation analysis on a large number of postmortem brain samples revealed that certain possible miRNAs were consistent across all samples in various comparisons. The potential miRNAs were further validated on APP transgenic (Tg2576), Tau transgenic (P301L) and wild type mice. MiRNAs were categorized as synaptosomal or cytosolic based on their existence and/or expression levels. In comparison to healthy controls, synaptosomal and cytosolic miRNAs were found to be dysregulated in AD patients. Further, Gene Ontology Enrichment and Ingenuity Pathway Analysis of synaptosomal miRNAs showed the involvement of these miRNAs in nervous system development, cell junction organization, and synapse assembly formation.
Conclusions: This is the first study to distinguish synapse miRNA candidates from cytosolic miRNAs based on their localization and expression levels and their possible roles in AD. **Key words:** Synapse, MicroRNAs, Synaptosomes, Cytosol, Synaptic activity

Disclosures: E. Orlov: None. **P. Gowda:** None. **P.H. Reddy:** None. **S. Kumar:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.04

Topic: B.07. Synaptic Plasticity

Support: NSF NN1-1707356
NSF NN2 - 2014862

Title: Presynaptic vesicles can provide membrane to enlarge boutons following LTP

Authors: *L. KIRK[1], D. HANKA[1], K. ZATYKO[1], T. M. BARTOL, JR[3], T. J. SEJNOWSKI[4], K. M. HARRIS[2];
[2]Ctr. for Learning and Memory, [1]Univ. of Texas at Austin, Austin, TX; [3]CNL-S, [4]Salk Inst., La Jolla, CA

Abstract: Long term potentiation (LTP) is the sustained increase in evoked response following the delivery of high frequency stimulation. In the hippocampus, LTP is the cellular correlate of learning and memory. There are many ultra-structural changes that occur at hippocampal synapses several hours after LTP. Most of this work has focused on changes in postsynaptic structures. However, less is known about presynaptic structural changes. We showed that two hours following LTP there is a significant drop in the reserve pool of synaptic vesicles, with a larger drop occurring at boutons that contain mitochondria. Previous work demonstrated that boutons are enlarged following LTP (Chereau et al., 2017). We hypothesized that the vesicles lost following LTP contribute their membrane to the enlarged boutons. Serial section electron microscopy was used to reconstruct presynaptic boutons and their components from CA1 stratum radiatum in hippocampal slices that received either control or TBS resulting in LTP. We expanded the suite of Neuropil Tools, built in the Blender environment, to measure surface areas of presynaptic boutons. We found that as the nondocked presynaptic vesicle pool decreased 2 hours following LTP, presynaptic boutons were enlarged with substantial increase in membrane surface area. Furthermore, the membrane from approximately 50% of the lost vesicles was sufficient to account for the growth in bouton surface area. We have analyzed the inter-bouton membrane area and found that its expansion was not sufficient to account for the rest of the vesicle membrane. Hence, we are currently analyzing other internal membrane bound organelles (mitochondria, SER, and endosomes) within the bouton to determine where the other 50% of vesicle membrane surface area may have gone.

Disclosures: L. Kirk: None. **D. Hanka:** None. **K. Zatyko:** None. **T.M. Bartol:** None. **T.J. Sejnowski:** None. **K.M. Harris:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.05

Topic: B.07. Synaptic Plasticity

Support: NWO (FOM) #16NEPH05

Title: Dendritic signaling pathways underlying endocannabinoid-mediated inhibitory bouton growth

Authors: *L. J. HERSTEL, H. HU, D. L. H. KRUIJSSEN, C. J. WIERENGA;
Cell Biology, Neurobio. and Biophysics, Dept. of Biol., Utrecht Univ., Utrecht, Netherlands

Abstract: Coordination between excitation and inhibition is essential for proper functioning of neuronal circuits. Inhibitory synapses can locally control the activity of excitatory inputs by their constructive placement on the same dendrite. We recently discovered that excitatory and

inhibitory plasticity is regulated at the synaptic level. By repeated stimulation of excitatory synapses, the formation of a nearby inhibitory bouton onto the same dendrite can be triggered. Our findings show that this process is mediated by the production of the endocannabinoid 2-arachidonoylglycerol (2-AG) which activates axonal cannabinoid type 1 (CB1) receptors. Spine stimulation failed to induce inhibitory bouton growth in presence of the cannabinoid receptor type 1 (CB1R)-antagonist AM251. In addition, we found that excitatory synaptic plasticity was affected by manipulations of the endocannabinoid system. Structural long-term plasticity of excitatory synapses was completely abolished by increasing 2-AG concentration via preventing degradation, whereas average spine growth was accelerated after AM251 application. These results might indicate a role for postsynaptic CB1 receptors in mediating excitatory synapse strengthening. We also explored the local dendritic signaling mechanism that underlies this coordinated inhibitory plasticity. We found that N-methyl-D-aspartate receptor (NMDA) receptor activation is required, but not sufficient, to induce bouton growth. We hypothesized that additional activation of metabotropic glutamate receptor 5 (mGluR5) is necessary. We used two-photon microscopy to image crossings between CA1 pyramidal cell dendrites (labeling via a patch pipette) and GFP-labeled GABAergic axons in organotypic hippocampal slices of GAD65-GFP mice (of both sexes, dissected at P6-8, used at DIV10-21). We stimulated multiple dendritic spines close (<10 um) to the crossing axon using two-photon glutamate uncaging (30x @0.5Hz) paired with somatic postsynaptic depolarization (0 mV, 100 msec). Pharmacological blockade of mGluR5 throughout the experiment with 2-Methyl-6-(phenylethynyl)pyridine (MPEP) did not prevent bouton growth, indicating that inhibitory bouton growth is independent of mGluR5 activation. Our results help to further unravel the activity-dependent signaling pathways that regulate coordination between excitatory and inhibitory synapses within dendrites.

Disclosures: L.J. Herstel: None. **H. Hu:** None. **D.L.H. Kruijssen:** None. **C.J. Wierenga:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.06

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 1 R15 NS101608-01A1

Title: Rab11 activity is required for proper localization of FasII and PIP2 at glutamatergic synapses

Authors: *I. SMITH[1], J. PRESTON[2], F. L. LIEBL[2];
[1]Southern Illinois Univ. Edwardsville, East Alton, IL; [2]Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Neural plasticity is an activity-dependent process that relies on vesicular transport of proteins like cell adhesion molecules (CAMs) and endocytic proteins at the synapse. Rab GTPases are a family of proteins that are critical to the membrane trafficking of vesicles. In particular, Rab11 plays a role in trafficking proteins from the recycling endosome to the plasma membrane thereby influencing synaptic localization of synaptic proteins. We have found that when Rab11 is constitutively active in neurons, there is an increase in synaptic Fasciclin II (FasII) and a decrease in the colocalization of Endophilin A (EndoA) and Dynamin, two endocytic proteins. In contrast, when a dominant negative Rab11 is expressed in neurons, we observe the opposite phenotype. In order construct a more comprehensive model of Rab11 function in neurons, we knocked down Rab11. While we found no significant changes in protein levels of FasII, EndoA, Glutamate Receptor IIC (GluRIIC), and the active zone protein Bruchpilot, there was a significant reduction in synaptic Phosphatidylinositol 4,5 biphosphate ($PI(4,5)P_2$) levels. Collectively, these data will deepen our understanding of how Rab11 may contribute to the synaptic vesicle cycle and synaptic plasticity.

Disclosures: I. Smith: None. **J. Preston:** None. **F.L. Liebl:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.07

Topic: B.07. Synaptic Plasticity

Support: NIH 5RO1NS112492
NIH R37MH070000

Title: The retrotransposon copia at the Drosophila neuromuscular junction

Authors: *P. G. M'ANGALE, A. SIMKIN, A. LEMIUEX, T. THOMSON, V. BUDNIK; Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Copia is an abundant retrotransposon found in *Drosophila melanogaster*. It is found as a full length or spliced form and we hypothesize that copia may play a significant role at the Drosophila neuromuscular junction. Copia was found enriched together with Arc1 in extra-cellular vesicles derived from S2 cells. From RNA-sequencing data coupled with digital PCR, we determined that copia has several full-length forms that are found in differing abundancy in the Drosophila larval brain or body wall muscles. We also show that, in the Drosophila larval brain, the short-spliced form (copia Gag) is highly abundant compared to the full-length version. From bacterially expressed copia Gag, we found that the resultant capsids were heterogeneous in size with most capsids within the 60 to 100 nm range. We will perform RIP-seq, or RT-qPCR to

establish the interactome of copia and perform electrophysiological recordings in copia RNAi flies to establish the physiological role of this retrotransposon.

Disclosures: P.G. M'Angale: None. **A. Simkin:** None. **A. Lemiuex:** None. **T. Thomson:** None. **V. Budnik:** None.

Poster

P120. Structural Plasticity: Presynaptic

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P120.08

Topic: B.07. Synaptic Plasticity

Support: UW Bridge Grant
UW Tacoma Scholarship & Teaching Fund

Title: Three-dimensional analysis of excitatory synapses in CA1 hippocampus reveals distinct populations of synaptic spinules and excitatory presynaptic boutons

Authors: A. YURINA, A. GORE, A. YUKEVICH-MUSSOMELI, ***M. NAHMANI**;
Univ. of Washington – Tacoma, Tacoma, WA

Abstract: Synaptic spinules are thin finger-like projections from one neuron that become fully embedded within the presynaptic or postsynaptic compartments of another neuron. While spinules are an evolutionarily conserved and relatively common feature of synapses across the animal kingdom, their specific function(s) remain unknown. This lack of understanding for what spinules “do” is mostly a reflection of their small size and the consequent difficulty in quantifying their abundance and 3D ultrastructure using previously available techniques. Recent focused ion beam scanning electron microscopy (FIBSEM) image volume analyses have demonstrated that spinules are embedded within ~ 25% of excitatory boutons in mammalian primary visual cortex (V1), yet the diversity of spinule sizes and ultrastructural relationship to their boutons across brain regions remain unclear. To begin to uncover the function of synaptic spinules, we sought to determine the abundance, origins, and 3D ultrastructure of spinules embedded within excitatory presynaptic boutons in mammalian CA1 hippocampus and compare these boutons with synaptic boutons bereft of spinules. Accordingly, we three-dimensionally reconstructed every excitatory presynaptic bouton (n = 138) along with their embedded synaptic spinules (if present; n = 122) and postsynaptic densities (PSDs, n=159) within a 5 nm^3 isotropic (7.68 x 10.24 x 5.33 µm) FIBSEM image volume from the CA1 hippocampus of an adult male rat. Surprisingly, we find that ~74% of excitatory boutons in this volume contain at least one spinule, suggesting that they are fundamental components of excitatory synapses in CA1. Moreover, synaptic spinules can be clearly differentiated into two groups: small clathrin-coated spinules, and 18 - 42X larger spinules without clathrin. Our analyses also reveal that a majority

of synaptic spinules in CA1 emanate from adjacent axons (60.5%), postsynaptic spines (38.1%), and adjacent (non-synaptic) spines (24.5%), with little variation in the volume, surface area, or bouton penetration distance across these spinule types. Yet interestingly, spinule-bearing boutons (SBBs) are 2.5X larger in volume and have 1.6X larger PSDs than boutons without spinules (non-SBBs), suggesting that SBBs are more stable and active than their non-SBB counterparts.

Disclosures: A. Yurina: None. **A. Gore:** None. **A. Yukevich-Mussomeli:** None. **M. Nahmani:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.01

Topic: B.13. Neuro-Oncology

Support: NPRP 11S-1214-170101 (Qatar National Research Fund)

Title: Curcumin's mechanistic effects on neuroblastoma and glioblastoma

Authors: *K. ZHAI[1], M. SHAKIBAEI[2], D. BUSSELBERG[1];
[1]Physiol. and Biophysics, Weill Cornell Medicine-Qatar, Doha, Qatar; [2]Ludwig-Maximilian Univ. Munich, Munich, Germany

Abstract: Curcumin, a major constituent of the turmeric (*Curcuma longa*) plant, is a polyphenolic compound with antioxidant, neuroprotective, and anticancer activities. As a natural compound with the potential to overcome the challenges of conventional anticancer therapies, curcumin was clinically trialed as a supportive therapy in several types of common malignancies. However, studies on curcumin and cancers of the nervous system remain sparse. Two such cancers are glioblastoma (GBM), an astrocyte-derived central nervous system malignancy, and neuroblastoma (NB), a peripheral nervous system tumor derived from neural progenitor cells. As such, this study critically reviews the recent literature on curcumin’s mechanistic effects on GBM and NB. Preclinical results indicate that curcumin inhibits PI3K/Akt/mTOR-dependent proliferation, induces apoptosis, and reduces cell migration and invasion in GBM cells. Similarly, curcumin’s anti-NB effects are mediated through apoptotic induction, cell cycle arrest, and mitochondrial dysfunction. These anticancer activities underscore curcumin’s potential as a therapeutic agent in cancers of the nervous system. However, while curcumin is clinically safe, its bioavailability and blood-brain barrier permeability remain important considerations on the path toward clinical trials in GBM and NB.

Disclosures: K. Zhai: None. **M. Shakibaei:** None. **D. Busselberg:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.02

Topic: B.13. Neuro-Oncology

Support: NSERC Grant 401389 to J.L.
CFI Grant 037755 to J.L.

Title: Flavonoids of Cannabis sativa limit glioblastoma survival and invasion by inhibiting TrkB signaling

Authors: *J. HOLBORN, C. GAGE, B. ALURAL, A. WALCZYK-MOORADALLY, C. PERRIN, N. JONES, T. AKHTAR, J. LALONDE;
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Abstract: Glioblastoma multiforme (GBM) is the most common and aggressive type of adult brain cancer. These tumours can occur in any region of the central nervous system and average survival time of patients after diagnosis is less than two years. This poor prognosis is attributable to the fact that GBM cells can rapidly invade into brain, a feature that is very difficult to attack with current treatment options. Better understanding of the molecular basis of GBM invasion, as well as how this phenomenon could be neutralized without damaging surrounding healthy cells, is therefore critically needed to develop more effective therapies. Abnormal neurotrophin signaling via the Tropomyosin receptor kinase B (TrkB) receptor is increasingly seen as an important factor in various types of brain tumour, including GBMs, and accumulating evidence suggest that targeting this pathway may be a valid strategy to limit the growth, proliferation, and/or motility of aggressive cancer cells. While screening for novel modulators of receptor tyrosine kinase (RTK) signaling, our group unexpectedly found that cannflavins—a class of flavonoid molecules that accumulates in the *Cannabis sativa* (*C. sativa*) plant—can prevent TrkB activation. This surprising finding motivated us to closely examine the impact of cannflavin A and cannflavin B against GBM biology. Here, we first present evidence that each type of flavonoid limits TrkB activation by Brain-Derived Neurotrophic Factor (BDNF) in dissociated mouse cortical neurons and human immortalized GBM cells (A172 and U87). Next, we report *in vitro* cytotoxicity experiments showing that inhibition of TrkB signaling by cannflavins sharply decreases viability of GBM cells by promoting programmed cell death. Finally, we show that a low, sub-toxic concentration of cannflavins is sufficient to significantly decrease the capacity of GBM cells to invade in the Boyden Chamber and spheroid migration assays. Together, our results suggest that targeting TrkB receptors with cannflavins can produce desirable anticancer effects against GBM cells by limiting molecular processes that support invasion and survival.

Disclosures: J. Holborn: None. **C. Gage:** None. **B. Alural:** None. **A. Walczyk-Mooradally:** None. **C. Perrin:** None. **N. Jones:** None. **T. Akhtar:** None. **J. Lalonde:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.03

Topic: B.13. Neuro-Oncology

Support: UC Davis Center for Companion Animal Health 2020-5-G

Title: Microglia derived olfactomedin-like 3 promotes primary mouse brain endothelial cell angiogenesis

Authors: R. G. TOEDEBUSCH, L. M. JOSEPH, C. A. LUCCHESI, L. A. WITTENBURG, ***C. TOEDEBUSCH**;
Univ. of California, Davis, CA

Abstract: Microvascular proliferation is a hallmark feature of glioblastoma multiforme (**GBM**), a uniformly lethal brain tumor. To date, anti-angiogenic therapies have targeted vascular endothelial growth factor (**VEGF**), the most abundant angiogenic factor in GBM. However, anti-VEGF therapy has proven ineffective for mitigating neoangiogenesis and improving patient survival, suggesting that VEGF-independent mechanisms of angiogenesis contribute to GBM progression. In recent years, a novel secreted protein, olfactomedin-like 3 (**OLFML3**), has been shown to promote tumor progression of several non-CNS cancers via increased angiogenesis. Preliminary data from our laboratory has demonstrated *Olfml3* expression in mouse brain endothelial cells (**EC**s) and microglia, the most abundant tumor invading immune cell type. However, the effect of exogenous OLFML3 and endogenous *Olfml3* on mouse brain EC angiogenesis remains unknown. Therefore, this study aimed to test the following two hypotheses: 1) Microglia-derived OLFML3 promotes primary mouse brain EC angiogenesis, and 2) EC-derived *Olfml3* is required for angiogenesis. To determine if OLFML3 was sufficient to induce angiogenesis, we exposed primary mouse brain ECs to human recombinant OLFML3 (**rhOLFML3**). rhOLFML3 (10 ng/mL) increased several phases of angiogenesis, including EC proliferation (CellTiter Glo; $p<0.0001$), migration (Boyden Chamber; $p=0.0375$) and differentiation tested via tube formation assay (total tubes, $p=0.009$; loops, $p=0.0011$; branching points, $p=0.0022$) relative to mock treated cells. To elucidate the contribution of microglia derived OLFML3, ECs were exposed to conditioned medium (**CM**) from immortalized isogenic control or *Olfml3* knockout microglial cells. All phases of angiogenesis were reduced in ECs exposed to CM from *Olfml3* knockout microglia (proliferation, $p=0.0035$; migration, $p=0.0016$; differentiation: total tubes, $p=0.0421$; branching points, $p=0.0467$). To determine if endogenous *Olfml3* is required for angiogenesis**,** primary ECs were transfected with a control, non-targeting,

or *Olfml3*-targeted siRNA which resulted in a 60% reduction in *Olfml3* mRNA. Proliferation (p=0.4773), migration (p=0.1430), and differentiation (total tubes, p=0.1431; loops p=0.1687; branching points pp=0.1788) were not altered following knockdown of EC *Olfml3*. Together, these data demonstrate that exogenous OLFML3 promotes primary mouse brain EC angiogenesis, but EC derived *Olfml3* may not be necessary. Importantly, microglial-derived *Olfml3* may be necessary for microglia-induced neoangiogenesis, providing a complementary, precise target for GBM patients.

Disclosures: R.G. Toedebusch: None. **L.M. Joseph:** None. **C.A. Lucchesi:** None. **L.A. Wittenburg:** None. **C. Toedebusch:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.04

Topic: B.13. Neuro-Oncology

Support: T32GM007367
F30CA257768

Title: In-vivo two photon imaging reveals the modulatory effects of mtor inhibition on neuronal activity at the infiltrative glioma margin

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Abstract: Recent studies have revealed that crosstalk between glioma cells and the brain microenvironment is a crucial regulator of cancer initiation and progression. We previously described a glioma model in Thy1-GCaMP6f mice that shows spontaneous seizure activity at the infiltrative margins of the tumor. Here, we used 2-photon imaging in awake Thy1-GCaMP6f mice to further characterize neuronal activity at the single cell level, both spontaneously and during whisker stimulation. AZD8055, a competitive dual mTORC1/2 inhibitor, has been shown to not only be a key regulator of neuronal excitability but also have anti-tumor effects in mouse models of glioblastoma. We have previously shown that AZD8055 suppresses neuronal hyperactivity in acute slices of this tumor model, yet little is known of its direct in-vivo effect on neuronal activity at the glioma border. At surgery, Thy1-GGaMP6f mice had glioma cells containing an mCherry fluorophore injected below the cortical surface prior to implantation of the cranial window. To investigate the impact of mTORC1/2 inhibition on neuronal activity,

imaging was performed before and after gavage of AZD8055 100mg/kg. In our imaging fields at the tumor border, we observed an intermingling of tumor cells and excitatory neurons. Our studies revealed that neuronal activity, as measured by thresholded peaks of somatic calcium events, is increased in response to whisker stimulation. Administration of AZD8055 reduced the rate of somatic calcium events in both the whisker stim and no stim conditions. To better understand how neuronal activity changes as a function of distance to tumor cells in the local microenvironment we utilized tumor cell mCherry signal to calculate a tumor burden score for each neuron. Our analysis revealed the rate of somatic calcium events increased as neuronal tumor burden scores increased, in both whisker stimulation and no stimulation conditions. Furthermore, this analysis illustrated that AZD8055 administration blunted this relationship. Lastly, we observed focal discharges of high amplitude synchronous calcium events, less than $500um^2$ in space and less than 1 second in time. These discharges were largely composed of neuropil fluorescence and were spatially concentrated to the high tumor burdened areas. Our ongoing analyses are aimed at further understanding the effects of mTOR inhibition on glioma-induced alterations in neuronal activity, neuronal synchrony, and whisker stimulus-evoked responses at the tumor margin. This study revealed that glioma cells have very local effects on neuronal activity at the infiltrative margin, and that these effects can be modulated by inhibition of mTOR signaling.

Disclosures: A.R. Goldberg: None. **A. Dovas:** None. **P.W. Calvaresi:** None. **H.T. Zhao:** None. **E.M. Merricks:** None. **A. Razavilar:** None. **W. Xu:** None. **N. Humala:** None. **A. Mahajan:** None. **M. Banu:** None. **S.K. Benson:** None. **E.M. Hillman:** None. **C.A. Schevon:** None. **D.S. Peterka:** None. **P. Canoll:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.05

Topic: B.13. Neuro-Oncology

Support: National Science Foundation RII Track-2 FEC 1539068
Presbyterian Health Foundation
Institute for Biomedical Engineering, Science and Technology at the University of Oklahoma

Title: Evaluation of fMRI Resting-State Functional Connectivity Following Resection of Brain Gliomas

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Abstract: Surgical resection is a mainstay of human glioma treatment. Extent of resection positively correlates with overall and progression-free survival, but resecting tumor-infiltrated brain tissue carries risk of inducing neurological deficits. However, it is increasingly recognized that the functional anatomy of the human neocortex is plastic. Functional connectivity of perilesional regions compared to that of corresponding contralateral regions has been implicated as a predictor of neurological outcome (Tarapore et al., 2012). Resting-state functional magnetic resonance imaging (fMRI) is an effective tool for identifying connectivity between tumor tissue and specific eloquent regions of the brain (Wongsripuemtet et al., 2018). To further our understanding of using fMRI to guide tumor resection, we evaluated whether fMRI could assess altered functional connectivity of perilesional tissue after surgery. Resting state fMRI data were obtained from nine subjects following glioma resection. Data analysis was conducted following our prior protocol (Yuan et al., 2017), using customized AFNI scripts. Spatial coregistration to anatomical images was performed for each fMRI dataset. The first five volumes of each run were excluded to allow the signal to reach steady state. Key processing steps were bandpass filtering (0.005-0.1 Hz), censoring data points with excessive motion (root mean square of 3D displacement > 0.3 mm) and smoothing using a Gaussian kernel (full width at half maximum = 6 mm). Functional connectivity of perilesional regions versus corresponding contralateral regions was evaluated via seed-based analysis, and further classified as having increased, decreased, or neutral connectivity. One subject was excluded due to excessive motion artifacts and another due to technical error resulting in echo-planar imaging data not covering the entire brain. In the remaining subjects, fMRI data allowed for identification of perilesional regions with altered connectivity compared to corresponding contralateral regions. In this study, we showed that fMRI resting state functional connectivity was able to identify regions of altered connectivity in patients following glioma resection, which is consistent with a previous report using magnetoencephalographic imaging (Tarapore et al., 2012). Future investigations could attempt to correlate the reorganization of functional connectivity to long-term neurological outcome. Patients may benefit from a more aggressive surgical strategy that accounts for predicted functional recovery after surgery. fMRI could become a valuable tool in the prediction and management of functional recovery.

Disclosures: A.J. Schroeder: None. **Y. Chen:** None. **T.M. Stephens:** None. **I.F. Dunn:** None. **H. Yuan:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.06

Topic: B.13. Neuro-Oncology

Support: NIH Grant R01ES007062-23

FIU Grant 800006850

Title: The Translocator Protein (TSPO) Genetic Polymorphism A147T is Associated with Worse Survival in Male Glioblastoma Patients

Authors: A. M. ACANDA DE LA ROCHA[1], K. M. TROIKE[4,5], T. J. ALBAN[4,5], M. M. GRABOWSKI[4,6], B. OTVOS[4,6], G. CIOFFI[8], K. A. WAITE[8], J. S. BARNHOLTZ-SLOAN[9,8], J. D. LATHIA[4,5,10,7], T. R. GUILARTE[1,2], *D. AZZAM[3];
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Abstract: Glioblastoma (GBM) is the most common primary brain tumor in adults, with few available therapies and a 5-year survival rate of 7.2%. Hence, strategies for improving GBM prognosis are urgently needed. The translocator protein 18kDa *(*TSPO) plays crucial roles in essential mitochondria-based physiological processes and is a validated biomarker of neuroinflammation, which is implicated in GBM progression. TSPO is located in the outer mitochondrial membrane and binds cholesterol through its C-terminal domain. One frequent single-nucleotide polymorphism (SNP) rs6971, which changes the alanine 147 into threonine (Ala147Thr) has been found in the C-terminal domain of the TSPO region and dramatically alters the affinity with which TSPO binds drug ligands. High *TSPO* gene expression is associated with reduced survival in GBM patients; however, the relation between the most frequent *TSPO* genetic variant and GBM pathogenesis is not known. The present study retrospectively analyzed the correlation of the *TSPO* polymorphic variant rs6971 with overall and progression-free survival in GBM patients using three independent cohorts. *TSPO* rs6971 polymorphism was significantly associated with shorter overall survival and progression-free survival in male GBM patients but not in females in a well curated Cleveland Clinic Foundation cohort of 441 patients. We observed similar trends in two other independent cohorts. These observations suggest that the *TSPO* rs6971 polymorphism could be a significant predictor of poor prognosis in GBM, with a potential for use as a prognostic biomarker in GBM patients. These results reveal, for the first time, a biological sex-specific relation between rs6971 *TSPO* polymorphism and GBM.

Disclosures: A.M. Acanda de la Rocha: None. **K.M. Troike:** None. **T.J. Alban:** None. **M.M. Grabowski:** None. **B. Otvos:** None. **G. Cioffi:** None. **K.A. Waite:** None. **J.S. Barnholtz-Sloan:** None. **J.D. Lathia:** None. **T.R. Guilarte:** None. **D. Azzam:** None.

Poster

P180. Neuro-Oncology: Molecular Mechanisms III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P180.07

Topic: B.13. Neuro-Oncology

Support: SHRF Establishment Grant
CoMRAD, College of Medicine, U of S.

Title: Autonomic nerve distribution predicts prostate cancer outcome

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Abstract: Recent studies demonstrated that autonomic nerves contribute to prostate cancer progression. For example, denser innervations of autonomic nerves are associated with high-grade tumors and a higher proliferation index. In particular, increased parasympathetic innervation was shown to promote disease recurrence. However, there is no data available on the spatial and temporal distribution of sympathetic and parasympathetic fibers, and their relation to each other, in prostate tumors, which is critical to understand their individual contribution to cancer and stromal cell metabolism. In this study, we examined the differential distribution of sympathetic and parasympathetic nerves in eight clinical samples of prostate tumors and corresponding adjacent normal tissues and correlated the findings with the associated clinical data. The samples were procured from Alberta Cancer Research Biobank following the guidelines approved by the Biomedical ethics committee. We used tyrosine hydroxylase and choline acetyltransferase staining to distinguish sympathetic and parasympathetic fibers, respectively, in these samples. We also determined the relative distribution of adrenergic beta and cholinergic muscarinic (M) receptors in these clinical samples. Our initial analysis revealed that higher sympathetic nerve density at the tumor site is associated with the development of metastasis. We also found a differential distribution pattern for adrenergic beta receptors. For example, beta 2 is highly expressed in cancer cells, while both beta 2 and beta 3 are expressed in stromal cells, indicating that sympathetic nerves promote both cancer and stromal cell metabolism. We found higher expression of M3 receptors in cancer cells while the tumor microenvironment showed the expression of both M1 and M3. Similar to sympathetic nerves, parasympathetic nerves were also abundant in high-grade tumors. Altogether, our result suggests that individual distribution of sympathetic and parasympathetic nerves and their receptors may have prognostic applications in prostate cancer. Detailed studies using a large set of samples are warranted for further validation.

Disclosures: S. Dwivedi: None. **J. Hall:** None. **S. Shrestha:** None. **T. Chaphekar:** None. **M. Bautista:** None. **A. Krishnan:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.01

Topic: C.01. Brain Wellness and Aging

Support: Institutional funds from Penn State College of Medicine

Title: Physical activity is associated with changes in functional connectivity metrics across the lifespan

Authors: *V. KUMAR, S. LACEY, K. SATHIAN;
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Abstract: Physical activity has been associated with improved brain health and cognitive function across the lifespan. However, this characterization has focused largely on describing the effects of physical activity in maintaining the structural integrity of the aging brain. Consequently, its impact on functional connectivity (FC) that reflects information transfer amongst distributed neuronal assemblies, and underlies complex cognitive functions, remains unclear. Here, we examined the relationship between levels of physical activity and FC patterns in resting-state magnetoencephalography (rs-MEG) and resting-state functional magnetic resonance imaging (rs-fMRI) data across the lifespan. Physical activity and energy expenditure (PAEE), rs-MEG, and rs-fMRI data of 540 participants were obtained from the Cambridge Centre for Ageing and Neuroscience repository (http://www.mrc-cbu.cam.ac.uk/datasets/camcan/). In rs-MEG data, we investigated FC dynamics using the global coherence metric that quantifies the degree of synchrony amongst distributed neural oscillations. Two-way ANOVA with factors of age (7 levels: age deciles from 18-88 years) and PAEE (2 levels: High and Low) revealed significant effects of both age [$F(6,539) = 8.92$, $p < 0.001$] and physical activity [$F(1,539) = 6.48$, $p = 0.01$] on α-band (8-12 Hz) global coherence, with no significant interaction [$F(6,526) = 1.05$, $p = 0.389$]. Overall, the low physical activity group showed higher α-band global coherence than the high physical activity group. In rs-fMRI data, we evaluated the graph-theoretic measure of network segregation i.e., the degree to which networks share connections among one another. We constructed a functional connectivity matrix based on a 264-node functional atlas (Power et al., Neuron, 72:665-678). Mediation analysis revealed that physical activity positively mediated the age/network-segregation relationship for the default-mode network [$\beta = 0.0003$, $p_{fdr} = 0.024$] and the fronto-parietal task network [$\beta = 0.0003$, $p_{fdr} = 0.009$] but negatively mediated the ventral attention network [$\beta = -0.0002$, $p_{fdr} = 0.05$]. Our results suggest that physical activity affects the processes that manifest in α-band global coherence and impacts the within- and between-network correlations that are characteristic of segregated and specialized organization. Furthermore, our findings indicate that FC metrics indexed by rs-MEG and rs-fMRI are sensitive indicators of the brain's response to physical activity.

Disclosures: V. Kumar: None. **S. Lacey:** None. **K. Sathian:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.02

Topic: C.01. Brain Wellness and Aging

Support: US NIH / NIA R56 AG060608; P30AG028383

Title: Sex differences in age-related false alarm and differential temporal and frontal responses during non-emotional working memory retrieval

Authors: *S. STROTHKAMP[1], L. BROSTER[2], B. AGHAYEEABIANEH[2], A. ANDERSON[2], D. K. POWELL[2], G. JICHA[1], Y. JIANG[2];
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Abstract: Inaccurate and prolonged working memory retrieval is hallmark of mild cognitive impairment (MCI) induced by Alzheimer's disease (AD) and related dementia pathologies. Increased false memory retrieval has been reported in normal cognitive aging (e.g. thinking a car in a parking lot is your car when it is not). It is unclear why there are more men in the US suffering from MCI (early stage of dementia), while AD disproportionally affect women. Here we test the hypothesis that sex differences in brain aging have differential effects on brain volumes and network associated working memory performance in preclinical cognitively intact older adults.
Forty-four (44) cognitively normal older adults (25 women; mean age 77), from the University of Kentucky ADC cohort, participated in the Bluegrass Working Memory Task inside the 3T Siemens magnetic resonance imaging (MRI) Scanner. The task is a 11-min modified delayed match-to-sample task (Jiang et al., 2016). A false alarm was defined as identifying a non-match visual object as a match target held in memory. MRI data was analyzed using Free Surfer. Spearman correlation and linear regression analyses were performed to test the hypothesis.
The mean false alarm rate in males was 3.74%, and 4.03% in females (no sex differences). Mean reaction time during false alarms (FA_RT) in men was 657 ms, and in women 792 ms (no sex differences, $p>0.05$). False alarm rate was negatively correlated with brain volumes in frontal and temporal regions. We observed significant correlations in FA_RT in all subjects in frontal and temporal regions. However, when subjects were separated by sex, FA_RT in males significantly correlated positively with frontal regions (e.g. rostral middle frontal gyrus and fontal pole) only while FA_RT in females correlated positively with temporal regions (e.g. transverse temporal gyrus).
We found that cognitively healthy older men and women made similarly rate of false alarm in working memory retrieval. However, the older men were significantly faster than women in false memory retrieval and involves differential cortical mechanisms. False alarm memory retrieval is

associated with decreased frontal volumes in older men but decreased temporal volumes more often in older women.

Disclosures: S. Strothkamp: None. **L. Broster:** None. **B. Aghayeeabianeh:** None. **A. Anderson:** None. **D.K. Powell:** None. **G. Jicha:** None. **Y. Jiang:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.03

Topic: C.01. Brain Wellness and Aging

Support: NIH NIMH R01MH118203
NIH NIMH U01MH121260
NIH NIMH R01MH096875
NIH NIA R01AG060931
NIH NIA R00AG051764)
NSF BCS 1800558
NSF BCS 1752393

Title: Links to disease and the social environment in the aging brain transcriptome

Authors: *K. L. CHIOU[1], A. R. DECASIEN[2], C. TESTARD[3], C. H. SPURRELL[6], A. A. GOGATE[7], H. A. PLINER[6], A. MERCER[8], J. E. NEGRÓN-DEL VALLE[1], M. C. JANIAK[9], S. E. BAUMAN SURRATT[10], O. GONZÁLEZ[11], N. R. COMPO[10], M. K. STOCK[12], A. V. RUIZ-GONZALEZ[10], M. I. MARTINEZ[10], A. D. MELIN[13], S. C. ANTÓN[2], C. S. WALKER[14], J. SALLET[15], L. M. STARITA[8], J. SHENDURE[8], J. P. HIGHAM[2], L. J. N. BRENT[16], M. J. MONTAGUE[4], M. L. PLATT[5], N. SNYDER-MACKLER[1];
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Abstract: Aging is a major risk factor for neurodegenerative diseases that impose tremendous burdens on people and societies. To understand trajectories of neurological aging in a primate, we generated one of the most comprehensive brain transcriptional datasets to date in a unique population of free-ranging, behaviorally phenotyped rhesus macaques (*Macaca mulatta*). We profiled bulk-tissue transcriptomes from 15 brain regions of 36 adult macaques (20 female, 16

male) and profiled single-nucleus transcriptomes from the dorsolateral prefrontal cortex (dlPFC) of 24 adult females spanning the natural age distribution. We then modeled transcriptomic data using linear mixed models that control for genetic relatedness among animals while controlling for sex and other technical and biological variables. We demonstrate that age-related changes in both the level and variance of gene expression occur in genes previously associated with neural functions and neurological diseases. Genes that are differentially expressed in diagnosed cases of Alzheimer's disease, for instance, are significantly associated with genes that change in both the mean and variance of expression in older ages. We also show that, of 8 distinct cell types, oligodendrocytes are the only cell type to significantly increase in proportion with age in the dlPFC. We identified, however, numerous cell-type-specific changes in expression, including decreases in expression of synaptic signaling-related genes in excitatory neurons, decreases in expression of genes regulating the modulation of excitatory postsynaptic potential in astrocytes, and increases in expression of genes related to proinflammatory responses in microglia. Finally, given existing evidence showing that elevated disease and mortality risk are caused in part by social adversity, we test if macaque dominance ranks—a proxy for social adversity—accelerate the effects of aging in the brain. We show that higher social status in females is associated with younger relative transcriptional ages, providing a link between the social environment and aging in the brain. Taken together, our findings lend insight into biological mechanisms underlying brain aging and indicate promising directions for improving social gradients in neurological health

Disclosures: K.L. Chiou: None. **A.R. DeCasien:** None. **C. Testard:** None. **C.H. Spurrell:** None. **A.A. Gogate:** None. **H.A. Pliner:** None. **A. Mercer:** None. **J.E. Negrón-Del Valle:** None. **M.C. Janiak:** None. **S.E. Bauman Surratt:** None. **O. González:** None. **N.R. Compo:** None. **M.K. Stock:** None. **A.V. Ruiz-Gonzalez:** None. **M.I. Martinez:** None. **A.D. Melin:** None. **S.C. Antón:** None. **C.S. Walker:** None. **J. Sallet:** None. **L.M. Starita:** None. **J. Shendure:** None. **J.P. Higham:** None. **L.J.N. Brent:** None. **M.J. Montague:** None. **M.L. Platt:** None. **N. Snyder-Mackler:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.04

Topic: C.01. Brain Wellness and Aging

Support: Aging Research Center of Newfoundland - Graduate Student Fellowship Award (Stipend)
Memorial University of Newfoundland Graduate Student Support Stipend

Title: Enteric Microbial Metabolites Alter Lipid Composition and Metabolism in Long Evans Rat Brain and SHSY5Y Cell Line

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Abstract: It is recognized that the microbiota in host's gut communicate with the CNS impacting the neurolipidome and brain health. A proposed mechanism for this communication is short chain fatty acids (SCFAs), neuroactive metabolites from the fermentation of carbohydrates in the colon and caecum. The literature demonstrates that SCFAs are beneficial for improving host health but emerging evidence from our work suggest these enteric metabolites can cause neural inflammation associated with altered neurolipidome. We exposed Long Evans rats to an acute dose of SCFAs consisting of 500mg/kg of acetate, propionate, and butyrate (60:20:20 molar ratio) for 7 days via IP injection. After the injection period, animals were sacrificed, and frozen brain samples used to assess the neurolipidome using UHPLC-RPC30-HESI-HRAM/MS-MS and DESI-MS. To compare animal results to in vitro, SH-SY5Y neuroblastoma cells were treated with the same ratio of SCFAs in equivalent molarity. The cell lipidome was assessed using UHPLC-RPC30-HESI-HRAM/MS-MS, Flow Cytometry, STED, Cytation3, and Seahorse XF. We observed cells arrest in cell cycle progression, increased mitochondrial mass, hyperfusion and fragmentation, decreased respiration and ATP production, with SCFA induced alterations to the neurolipidome. Trauma/neurodegenerative diseases have been linked to modified lipid compositions within the brain. Alterations were observed in sphingolipids, phospholipids, plasmalogens, neutral lipids, and mitochondrial lipids in both rat brain and cell line. Both increases and decreases in phospholipids included saturated (16:0,18:0), monounsaturated (16:1,18:1), and polyunsaturated (20:4,22:6) fatty acid lipid composition. These alterations have the potential to affect lipid membrane stability, fluidity, and inflammation. This inluded plasmalogen species, which are important in oxidation and oxidative stress. Triglycerides were significantly impacted by the treatment resulting in a lowered concentration. While considered for fat storage and energy only, recent research has been shown that TGs can impact cognition within the brain, making them now relevant to diseases such as Alzheimer's disease. Observed alterations in mitochondrial specific lipids cardiolipin and acyl-carnitine can have energy and functional implications that are connected to neurological disorders within the brain. This study aims to understand how metabolites change the lipid composition within brain. This could lead to an understanding of how susceptible an organism may be to neurological health consequences or disorders following acute exposure to SCFAs.

Disclosures: T.A. Fillier: None. **S. Shah:** None. **K. Doody:** None. **T.H. Pham:** None. **I. Aubry:** None. **M.L. Tremblay:** None. **S. Cheema:** None. **J.J. Blundell:** None. **R.H. Thomas:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.05

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant K99AT010158
NIH Grant 5 P30 AG066518-02
NIH Grant UL1TR000128

Title: Yogic Breathing and Cerebrospinal Fluid Dynamics

Authors: *S. YILDIZ[1], J. GRINSTEAD[5], A. HILDEBRAND[2], J. N. OSHINSKI[6], W. D. ROONEY[3], M. M. LIM[7], B. S. OKEN[4];
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Abstract: Cerebrospinal fluid (CSF), a clear colorless fluid, is one of the two discrete fluid compartments of the brain along with interstitial fluid (ISF). CSF, together with ISF, plays a key role for the removal of solutes and metabolic wastes from the brain interstitium. Regulation of CSF dynamics is crucial to optimize the central nervous system (CNS) health, and impaired CSF circulation can potentially contribute to CNS diseases such as Alzheimer's disease. Therefore, understanding the mechanisms driving CSF movement, and interventions that influence its resultant removal of waste products from the brain is of high scientific and clinical impact. Pulsatile CSF movement is driven by pressure changes in CNS vascular system due to cardiac pulsation and respiration. Since pulsatile CSF dynamics is sensitive and synchronous to respiratory movements, we are interested in identifying potential integrative therapies such as yogic breathing to regulate and enhance CSF dynamics, which has not been reported before. We investigated the pre-intervention baseline data from our ongoing randomized controlled trial studying the impact of long-term practice of yogic breathing on CSF dynamics. The purpose of this study is to study the immediate modulation of pulsatile CSF velocity waveforms at the level of foramen magnum during four yogic breathing practices versus spontaneous breathing using a non-invasive magnetic resonance imaging (MRI) approach. In examinations of 18 healthy participants (eight females, ten males; mean age 34.9 ± 14 (SD) years; age range: 18-61 years), we discovered immediate increase (16% - 28%) in cranially-directed velocities of instantaneous-CSF velocities during yogic breathing compared to spontaneous breathing, with most statistically significant increase during deep abdominal breathing (28%, p=0.0008). In addition, cardiac pulsation was the primary driver of CSF during all breathing conditions except during deep abdominal breathing when there was a comparable contribution of respiration and cardiac

pulsation, which suggests respiration can be the primary driver for pulsatile CSF depending on individual differences in breathing depth and location. Since increased pulsatile CSF dynamics is suggested to increase brain's waste clearance through increased mixing and diffusion, we hypothesize that a potential underlying mechanism for the benefits of sustained training yogic breathing is its impact on pulsatile CSF dynamics, in turn removal of solutes from the brain. Further work is needed to confirm this hypothesis in healthy young and aged brain, and neurological conditions such as Alzheimer's disease.

Disclosures: S. Yildiz: None. **J. Grinstead:** A. Employment/Salary (full or part-time):; Siemens Medical Solutions USA, Inc. **A. Hildebrand:** None. **J.N. Oshinski:** None. **W.D. Rooney:** None. **M.M. Lim:** None. **B.S. Oken:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.06

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant - AG067781, AG045571
NIA ADC Grant - AG013854

Title: Synaptic Integrity in Cognitive SuperAgers

Authors: R. TAEFI, K. HAYNES, I. A. AYALA, *E. TAEFI, C. B. SWOPE, M. E. FLANAGAN, T. GEFEN, E. ROGALSKI, M. M. MESULAM, C. GEULA;
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Abstract: Decline in memory is a normal characteristic of aging. However, there is an alternative trajectory that resists the cognitive changes of normal aging. Our center has been engaged in investigations of a subset of individuals over the age of 80 with performance on tests of episodic memory equal to or better than individuals 20-30 years their junior. We have used the term "SuperAger" to refer to such individuals. SuperAgers show larger cortical volumes, less ApoE4, more von Economo neurons, and fewer instances of Alzheimer's disease (AD) pathology than their cognitively average peers. Synaptic loss is one of the strongest correlates of cognitive decline in the normal elderly and in AD. The purpose of this study was to investigate whether greater synaptic integrity is a characteristic of SuperAger brains. The status of synaptic proteins in individuals with a range of cognitive performance was studied. We examined the levels of the presynaptic protein synaptophysin and the postsynaptic density protein 95 (PSD-95) in fresh frozen tissue homogenates of the middle frontal gyrus (MFG) and the superior temporal gyrus (STG) of SuperAgers, cognitively average elderly, and AD patients ($N \geq 3$ per group)

using Western blot analysis and specific antibodies. Optical densities of bands were determined using the Image J software, and synaptic protein levels were expressed as percentage of the housekeeping protein GAPDH. As expected, levels of synaptic proteins were decreased in AD brains when compared with controls (0-31%). Interestingly, results indicated higher levels of synaptophysin in MFG (21%), and of PSD-95 in STG (51%) in SuperAgers when compared with cognitively average elderly. While preliminary, these findings suggest that enhanced integrity of cortical synapses, assessed through levels of synaptic proteins, may contribute to the SuperAging phenotype.

Disclosures: R. Taefi: None. **K. Haynes:** None. **I.A. Ayala:** None. **E. Taefi:** None. **C.B. Swope:** None. **M.E. Flanagan:** None. **T. Gefen:** None. **E. Rogalski:** None. **M.M. Mesulam:** None. **C. Geula:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.07

Topic: C.01. Brain Wellness and Aging

Title: Increased Neural Heterogeneity after 30 minutes of Aerobic Exercise in Older Adults.

Authors: *J. PURCELL[1], J. WON[1], L. R. WEISS[1], A. ALFINI[2], D. CALLOW[1], J. C. SMITH[1]; [1]Univ. of Maryland, College Park, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Background: As one progresses into old age, there is a higher risk of decreased physical and cognitive health. Although there is a growing body of work demonstrating that aerobic exercise can help improve neural function, the specific mechanisms of improvement are not well understood, especially immediately after exercise. One hypothesized mechanism is that a single session of exercise can lead to increased local neural heterogeneity within brain regions important for separating neural signals (Suwabe et al. 2018) including the hippocampus and cerebellum (Cayco-Gajic and Silver 2019). To date, no study has examined differences in local neural heterogeneity following a single bout of exercise in older adults. **Hypothesis:** There will be relatively high levels of neural heterogeneity (i.e., less similarity of adjacent neural signals) after 30 minutes of aerobic exercise within both the hippocampus and cerebellum. **Methods:** 32 physically active, right-handed older adults (ages, 55-85 years) participated in a within subject randomized crossover design. We employed a novel analytic technique termed General Heterogeneity Regression which concatenates several distinct fMRI runs together (resulting in about 30 minutes of fMRI data per time point/subject) with the goal of producing a reliable and reproducible measure of local neural heterogeneity. We compared the whole brain neural heterogeneity after 30 minutes of moderate intensity aerobic exercise (stationary bike) relative to 30 minutes of rest. **Results:** Aerobic exercise was associated with higher heterogeneity in the left

hippocampus, bilateral lingual gyrus, left anterior cingulate, and cerebellum (bilateral Crus 1 and 2, bilateral vermis, right I-IV, and left VI), confirming our hypothesis. These results were both reliable (whole brain permutation-based threshold-free cluster enhancement corrected, two-way $p<0.05$), and replicable (split-half reproducibility intra-class correlations > 0.4). **Significance:** These findings provide support for the hypothesized mechanism that after just 30 minutes of aerobic exercise there are higher levels of activation heterogeneity within regions that play an important role in separating similar neural signal patterns. These findings further suggest that a single bout of aerobic exercise may lead to improvements on tasks for which pattern separation is important, such as associative learning (Cayco-Gajic and Silver 2019) and cognitive control (Abrahamse et al. 2016). Future work will investigate exercise induced changes in neural heterogeneity as a possible mechanism of improved neural health in older adults at risk for or with a neurodegenerative disease.

Disclosures: J. Purcell: None. **J. Won:** None. **L.R. Weiss:** None. **A. Alfini:** None. **D. Callow:** None. **J.C. Smith:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.08

Topic: C.01. Brain Wellness and Aging

Support: James S. McDonnell Foundation
Departmental Funds

Title: Neurophysiologic consequences of knocking out $GABA_A$ receptors containing the α4 subunit in mice

Authors: *P. GARCIA, T. Z. CASSIM, M. GRAVES, M. T. MANSOURI;
Columbia Univ. Med. Ctr., New York, NY

Abstract: Introduction: The GABA Type A receptor ($GABA_AR$) is important for mediating unconsciousness in clinical anesthesia. Monitoring of neurophysiologic signals via the electroencephalogram (EEG) during anesthesia can help identify characteristic patterns in sleep, general anesthesia, and coma. Burst suppression is a discontinuous EEG pattern typically seen with administration of excessive anesthesia but can also be observed in older patients that are receiving usual doses of analgo-sedative medication. Previously we have demonstrated subtle differences in burst features that depend of the anesthetic used (propofol vs sevoflurane). **Objective:** Here we examine the role of a specific subtype of $GABA_AR$ (α4-containing) in the relationship of drug choice and anesthetic dose with burst suppression. **Methods:** Age-matched, adult male (26-30 g) mice genetically manipulated to knock-out (KO) the *gabra4* gene and wild-

type (WT) littermates were used. Following the administration of isoflurane (1.5% - 2.5%, inhaled), propofol (150 mg/kg, intraperitoneal injection), ketamine/xylazine (K/X, 90/5 mg/kg, intraperitoneal injection) and ketamine (90 mg/kg, intraperitoneal), sub-dermal needle electrodes were applied and electrophysiology was recorded wirelessly using an OpenBCI™ Cyton board. The collected data were analyzed using custom scripts written in MATLAB. **Results:** We demonstrated that genetic ablation of GABRA4 results in discontinuous EEG patterns at a lower concentration of isoflurane than the WT control group. The results from intraperitoneal injection of other clinically used anesthetic agents was more variable. **Conclusion:** We conclude that there are important differences in thalamocortical neurophysiology mediated by α4-containing $GABA_A$Rs. These findings may be important to consider during the titration of analgo-sedative agents in clinical scenarios.

Disclosures: P. Garcia: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Columbia University owns patents on which I am a named inventor. **T.Z. Cassim:** None. **M. Graves:** None. **M.T. Mansouri:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.09

Topic: C.01. Brain Wellness and Aging

Title: Mediterranean diet adherence, gut microbiota, and Alzheimer's or Parkinson's disease risk: A systematic review

Authors: *R. J. SOLCH[1], J. O. AIGBOGUN[1], A. G. VOYIADJIS[1], G. M. TALKINGTON[1], R. M. DARENSBOURG[1], S. S. O'CONNELL[2], K. M. PICKETT[3], S. R. PEREZ[1], D. M. MARAGANORE[1];
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Abstract: INTRODUCTION: There are no known cures for the two most prevalent neurodegenerative diseases, Alzheimer's disease (AD) and Parkinson's disease (PD). The Mediterranean diet (MeDi) has been associated with neuroprotection, but the mechanism of this effect is unknown. These benefits may be in part due to downstream effects of the modulated gut microbial composition. This systematic review aims to explore the modulatory effects of adhering to a MeDi on the gut microbiota in relation to AD or PD risk. **METHODS:** Following the preferred reporting items for systematic review and meta-analysis guidelines, a search from inception to November 2020 was conducted in PubMed, CINAHL, EMBASE, Web of Science, Global Health, Biological Abstracts, and Grey Literature Report databases. As the topic of gut

microbiota, Mediterranean diet and AD or PD has not been extensively studied, two searches for human studies were conducted: 1) (MeDi or Microbiota) and (PD or AD) and 2) MeDi and Microbiota. Screening of abstracts and full-texts, and completion of data extraction and quality assessments were independently completed by two authors. **RESULTS:** Of the 4,672 studies identified, 65 were eligible and included in this systematic review. These studies were further divided into five groups: MeDi and AD risk (n=5), MeDi and PD risk (n=2), MeDi and microbial composition or metabolomics (n=21), microbial composition or metabolomics and AD (n=7), and microbial composition or metabolomics and PD (n=30). Generally, adherence to the MeDi was associated with a lower risk of AD development, but was conflicting for PD risk. Additionally, those adhering to a MeDi had shifts in short-chain fatty acid producing bacteria. These changes were supported by metabolomics data showing increases in at least one short-chain fatty acid. Further, MeDi-associated bacteria shared commonalities between AD or PD microbiota changes. Eight genera and two species had an inverse relationship with MeDi and AD, and one family, eight genera and three species had an inverse relationship with MeDi and PD. **CONCLUSION:** MeDi may be a neuroprotective modifiable lifestyle factor through its effects on the gut microbial composition. However, as these associations were made mostly from observational studies, more interventional studies are needed to expand further our understanding of the relationship between MeDi, gut microbiota and AD or PD risk.

Disclosures: R.J. Solch: None. **J.O. Aigbogun:** None. **A.G. Voyiadjis:** None. **G.M. Talkington:** None. **R.M. Darensbourg:** None. **S.S. O'Connell:** None. **K.M. Pickett:** None. **S.R. Perez:** None. **D.M. Maraganore:** None.

Poster

P190. Brain Wellness: Mechanisms and Biomarkers

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P190.10

Topic: G.04. Emotion

Support: NSERC USRA
Carleton University COVID-19 Rapid Response Research Grant

Title: Impact of early physical activity on feelings of depression during the COVID-19 pandemic

Authors: *N. K. KOZIEL LY[1], L. MOHAMUD[1], K. MATHESON[1,2], H. ANISMAN[1,2], M. J. CHEE[1];
[1]Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Royal Inst. of Mental Hlth. Res., Ottawa, ON, Canada

Abstract: Physical activity is important for promoting both metabolic and mental health. It is a vital aspect of body weight management, but it is also a form of active coping to manage stress, including that from lifestyle changes during the COVID-19 pandemic. While it is known that physical activity is related to reduced feelings of depression or anxiety, the objective of this study was to determine: 1) if physical activity at the onset of the pandemic predicted lower feelings of depression or anxiety over time, and 2) whether appraisals of stressful situations mediated this relationship. We surveyed 319 adults living in Canada or the United States during the COVID-19 pandemic to assess physical activity, stress appraisals, and mental health outcomes at two time points - May (T1) and October 2020 (T2). There was an overall decrease in physical activity at T2, which was attributed to a decrease in leisure-time physical activity (LTPA) but not on-the-job physical activity. Higher LTPA at T1 was associated with control-by-self appraisals of stressful situations (at T1 and T2), while lower initial LTPA was associated with appraisals of uncontrollability (at T1 and T2) and threat (at T2). Furthermore, lower initial LTPA was also associated with higher feelings of depression (at T2) but not anxiety. Hierarchical regression analyses demonstrated that stress appraisals of control-by-self, uncontrollability, and threat at T2 each uniquely contributed to feelings of depression at T2, over-and-above the contribution of T1 stress appraisals. Finally, while all three forms of stress appraisals at T2 were related to depression, only the appraisal of uncontrollability mediated the relationship observed between LTPA at T1 and depression at T2. In summary, physical activity during times of leisure in the early stages of the pandemic indirectly predicted subsequent feelings of depression 5 months later, reflecting the association between activity and being less likely to appraise stressful situations as uncontrollable. Although correlational, these data point to the possibility that physical activity may protect against worsened mental health outcomes during stressful periods like those associated with the COVID-19 pandemic.

Disclosures: N.K. Koziel Ly: None. **L. Mohamud:** None. **K. Matheson:** None. **H. Anisman:** None. **M.J. Chee:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.01

Topic: C.06. Neuromuscular Diseases

Support: R01 NS098819I
Target ALS
Johns Hopkins Discovery Fund

Title: Early cell-type-specific transcriptomic changes in motor cortex of a C9orf72 ALS/FTD mouse model

Authors: *A. SPIEGEL[1,2], K. LEE[4], J. W. ANDERSEN[5], L. A. GOFF[3,1,2], S. P. BROWN[1,2]; [1]Solomon H. Snyder Dept. of Neurosci., [2]Kavli Neurosci. Discovery Inst., [3]McKusick-Nathans Inst. of Genet. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Sch. of Dentistry, Seoul Natl. Univ., Seoul, Korea, Republic of; [5]Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Neurodegenerative diseases are characterized by the progressive loss of specific neuronal populations. In amyotrophic lateral sclerosis (ALS), for example, corticospinal and spinal motor neurons are particularly vulnerable to degeneration. Although genetic causes of ALS have been identified, the most common being a repeat expansion in the *C9orf72* gene, why certain cell types are particularly affected by these mutations remains poorly understood. We hypothesize that early functional changes in neurodegenerative diseases such as ALS are cell-type-specific, given the selective vulnerability of specific cell types in disease. We therefore asked whether we could identify cell-type-specific transcriptomic responses in a bacterial artificial chromosome (BAC) transgenic mouse model of *C9orf72* disease (Liu et al., 2016) during the first postnatal week. Specifically, this transgenic mouse model expresses the human *C9orf72* gene containing a hexanucleotide repeat expansion—a mutation that has been associated with ALS, frontotemporal dementia (FTD), and other neurodegenerative diseases. We performed single-cell RNA-seq on cells from the motor cortex from five wildtype and five *C9orf72* mice at P6-7. Using a generalized linear mixed model to identify genes differentially expressed across all cells with respect to genotype, we failed to identify any genes with differential expression. However, inclusion of cell type in this model revealed 220 genes differentially expressed in a cell-type-specific manner, including genes previously implicated in ALS. We further used nonnegative matrix factorization to identify patterns of gene expression that were differentially used with respect to genotype, again in a cell-type-specific manner. These analyses highlight the importance of transcriptomic analysis at single-cell resolution, enabling the detection of subtle cell-type-specific effects early in disease. They also provide a general workflow for identifying cell-type-specific signatures of disease in single-cell RNA-seq datasets.

Disclosures: A. Spiegel: None. **K. Lee:** None. **J.W. Andersen:** None. **L.A. Goff:** None. **S.P. Brown:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.02

Topic: C.06. Neuromuscular Diseases

Support: NSF Grant 1944247
AARG-2018-59104
Emory ADRC P30 AG066511

Title: In-silico multi-factorial system stability to identify promising SOD1-G93A ALS mouse combination treatments

Authors: *S. BI[1], A. KRUEGER[2], A. LEE[1], C. MITCHELL[1];
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Abstract: Superoxide dismutase 1 glycine 93 to alanine (SOD1 G93A) transgenic mice exhibit mutations associated with amyotrophic lateral sclerosis (ALS). However, it is difficult to do high throughput combination treatment testing for ALS in-vivo. The primary study objective is to model combination therapy performance for ALS in-silico using "stability", equating to homeostasis, as the criterion for a successful treatment. Computational models of wild-type (WT) and SOD1 G93A (ALS) mouse physiological stability were developed using dynamic meta-analysis, mathematical optimization, and unsupervised machine learning. WT and ALS models were based on seven prominent regulatory mechanisms in ALS pathophysiology: apoptosis, bioenergetics, chemistry, excitotoxicity, inflammation, oxidative stress, and proteomics. Each ontology was divided into 2 aggregation schemes with opposite physiological functions. For both models, the gain values or "modulatory feedback" linking each mechanism to the system of dynamical derivatives were optimized using a genetic algorithm and validated using a separate experimental dataset. To simulate ALS treatment and re-stabilization, 2548 different combinations of gain parameters representing three-way treatments were examined. Mathematically, stability was defined as a treatment combination for which all real portions of the eigenvalues of the gain matrix were negative. Three different optimization algorithms and varying modulation bounds were tested to determine the effect size necessary to re-stabilize the ALS system. Increased bounds for modulation of the gain values led to an increased number of stabilized combinations for all treatment optimization algorithms used. For maximum effect sizes of 500%, 1000%, and 1500%, a maximum of 10, 24, and 50 combinations stabilized, respectively. The combinations that stabilized at smaller effect sizes continued to stabilize for larger maximum effect sizes. Combinations modulating gain values corresponding to anti-apoptosis, energy consumption, energy production, anti-inflammatory, and pro-proteomic factors stabilized most often. The identified treatment combinations, stability patterns, and effect sizes provide critical insight for experimental development and testing of translational ALS treatments and etiological hypotheses.

Disclosures: S. Bi: None. **A. Krueger:** None. **A. Lee:** None. **C. Mitchell:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.03

Topic: C.06. Neuromuscular Diseases

Support: NIH RO1 NS091299
NIH R21 NS115514
Undergraduate Biology Research Program
Ronald E. McNair Achievement Program
HHMI Gilliam Fellowship

Title: Deciphering the mechanisms underlying the neuroprotective effect of glycolysis in ALS

Authors: *S. LOGANATHAN[1], H. E. BALL[1], M. E. MACIAS[1], M. L. TRAN[1], G. M. PETERSON[1], G. BIRCHAK[1,3], E. MANZO[1,4], D. C. ZARNESCU[1,2];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder for which there is no cure. ALS affects upper and lower motor neurons leading to loss of motor coordination, irreversible paralysis, respiratory failure and eventually death within 2-5 years of diagnosis. TDP-43 pathology is observed in &gt97% of ALS cases. Our lab has developed a *Drosophila* model of ALS based on overexpression of human TDP-43 that recapitulates multiple disease aspects including cytoplasmic aggregates, neuromuscular junction (NMJ) abnormalities, reduced lifespan, and locomotor defects. Our lab has shown that glycolysis upregulation, specifically an increase in Phosphofructokinase-1 (PFK1; PFK in *Drosophila*), provides a compensatory, neuroprotective effect in *Drosophila* models of ALS based on TDP-43 proteinopathy. In this study we aim to understand the mechanisms underlying the neuroprotective effect of PFK overexpression (OE) in ALS. Recent findings from other groups show that PFK forms clusters at the synapses of *C. elegans* where it is required for synaptic vesicle (SV) cycling, and along with other glycolytic enzymes, forms Glycolytic bodies in yeast under stress, which is associated with increased glucose consumption and cell survival. In addition, pyruvate, the end product of glycolysis, was found to be increased in multiple ALS models. Interestingly, pyruvate has also been shown to have strong Reactive Oxygen Species (ROS) scavenging capacity, protecting mitochondrial membrane potential, and regulating cell metabolism. These findings led us to hypothesize that 1) PFK localization and/or Glycolytic body assembly are altered at ALS synapses 2) PFK OE mitigates TDP-43 dependent SV cycling deficit at the larval NMJ in Drosophila and/or 3) PFK OE mitigates oxidative stress by antioxidant activity of pyruvate. We began to test this hypothesis by using expansion microscopy, which shows that PFK forms puncta within *Drosophila* NMJs, in the context of TDP-43 OE. Furthermore, PFK clustering is observed in Drosophila primary motor neurons with TDP-43 proteinopathy. In addition, we used FM1-43, a membrane probe, and found that PFK OE rescues TDP-43 induced SV cycling deficits. We are currently testing if oxidative stress is altered under the conditions of PFK overexpression in several ALS models. Together, these

results suggest that PFK forms puncta at the synapse and mitigates TDP-43 induced SV cycling deficits. These findings may uncover novel therapeutic strategies for ALS.

Disclosures: S. Loganathan: None. **H.E. Ball:** None. **M.E. Macias:** None. **M.L. Tran:** None. **G.M. Peterson:** None. **G. Birchak:** None. **E. Manzo:** None. **D.C. Zarnescu:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.04

Topic: C.06. Neuromuscular Diseases

Support: The National Academy of Sciences (award #: 2000009148)
NIH-NINDS (grant #: NS091836)
Science and Technology Development Fund (STDF) – US-Egypt Joint Science and Technology Research Collaboration Program (grant # USC18-1082)
The Google PhD fellowship program

Title: Spiking motoneurons effective connectivity as an early electrophysiological biomarker of amyotrophic lateral sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is thought to be a multi-factorial disease involving both autonomous and non-autonomous cell mechanisms. Of the many pathways under examination, abnormalities in motoneuron (MN) excitability remain the most tightly linked to disease pathogenesis. Experimentally, abnormalities were found in MN electrical behavior long before clinical symptoms manifestation, which motivates the development of machine learning and statistical approaches that could detect irregular patterns in MN firing compared to normal firing behavior. In this work, we use machine learning methods to infer the effective connectivity among individual MNs from their spiking activity and use that as an early electrophysiological biomarker of ALS. Specifically, cross-correlograms computed from pairs of same-type MNs are used to construct effective connectivity graphs in which nodes represent MNs and directed edges represent connections. A machine learning classifier is then applied to discriminate between graphs representing ALS versus Wild-Type (WT). A high-fidelity computational model of the spinal MN pool was used in this study to simulate the spiking activity of type-identified MNs (S,

FR, and FF cell types) under WT and ALS conditions. In the simulations, MNs were activated via synaptic inputs using trains of monophasic pulses. Our results demonstrate that effective connectivity among MNs changes early in ALS. Applying the machine learning classifier to the obtained feature space resulted in average classification accuracies of 97 ± 5.1%, 94 ± 6.9 % and 93 ± 7.7 % for the FF, FR and S types graphs, respectively. Our analysis reveals that the accuracies obtained for different cell types are consistent with the simulated ALS conditions depending on cell-type vulnerability. These results provide evidence that MN effective connectivity could serve as an electrophysiological biomarker for early ALS diagnosis.

Disclosures: A.Y. Abdelaal: None. **M.H. Mousa:** None. **M. Gamal:** None. **M.I. Khalil:** None. **S.M. Elbasiouny:** None. **S. Eldawlatly:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.05

Topic: C.06. Neuromuscular Diseases

Support: NIH- National Institute on Deafness and Other Communication Disorders, grant number R21 DC016071

Title: Exploring optogenetic stimulation of the hypoglossal nucleus to preserve tongue structure and function in a mouse model of ALS

Authors: *B. STEALEY[1], K. OSMAN[1], J. WANG[2], C. HAXTON[1], T. E. LEVER[3], I. OZDEN[2];
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Abstract: Impaired tongue function due to progressive degeneration of hypoglossal nucleus (HGN) neurons in amyotrophic lateral sclerosis (ALS) commonly progresses to dysphagia and increased risk of malnutrition, aspiration pneumonia, and feeding tube dependence. Importantly, there are currently no effective treatments to prevent or slow the progression of HGN degeneration. Here, we used a translational mouse model of ALS to develop a targeted excitatory optogenetic stimulation approach to preserve HGN neurons and prevent corresponding tongue atrophy and dysphagia.
We stereotaxically injected AAV5-hSyn-ChR2-eYFP (ChR2) into the HGN (4 WT, 4 SOD1-G93A mice) and implanted an optrode into the HGN 3 weeks post-injection. Opsin expression was confirmed in all mice by evoked HGN activity and tongue movement in response to optogenetic stimulation (40Hz, 50% duty cycle, 450nm). Tongue movements were also triggered by surface illumination of the tongue (6/8 mice), meaning the opsin spread to the distal

hypoglossal nerve. Post-mortem histological examination confirmed strong opsin expression in the HGN and hypoglossal nerve, thus demonstrating they can be reliably targeted for functional optogenetic stimulation.
Videofluoroscopic swallow studies (VFSS) revealed our surgical procedures had no significant effect on licking and swallowing function or survival ($p<0.05$) for WT or SOD1 mice compared to archived data from the same colony. To evaluate the effects of HGN optogenetic stimulation on behavior, we examined tongue function in real-time by assessing lick rate during voluntary drinking and monitoring local field potentials in the HGN. We found that lick rate can be modulated in a graded fashion by tuning the intensity of HGN optogenetic stimulation (n=4/4). We hypothesize that optogenetic stimulation alters lick rate by causing muscle stiffness; higher laser intensities generate more resistive force against tongue movement to cause slower licking. In conclusion, the HGN can be safely and reliably targeted for optogenetic stimulation. Optogenetic responses were strong and could evoke tongue movements. Therefore, optogenetic stimulation of the HGN holds promise as a targeted tongue resistance exercise program where the resistive force can be tuned by adjusting the laser intensity during drinking. Given that optogenetic stimulation has shown to promote axonal growth and proliferation in previous studies, we hypothesize controlled optogenetic stimulation of the HGN may significantly delay neuronal death and preserve licking /swallowing function, which if translatable, could prolong the survival of human ALS patients and enhance quality of life.

Disclosures: B. Stealey: None. **K. Osman:** None. **J. Wang:** None. **C. Haxton:** None. **T.E. Lever:** None. **I. Ozden:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.06

Topic: C.06. Neuromuscular Diseases

Title: Ripk1 kinase activation contributes to disease pathophysiology in als

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the spinal cord and brain. Based on pathological and genetic findings, ALS sits on a spectrum with frontotemporal dementia (FTD), a neurodegenerative disease that leads to the progressive degeneration of neurons in the temporal and frontal lobes of the brain.

Recently, several lines of evidence have suggested that Receptor interacting protein kinase 1 (RIPK1) activation may be causally linked to the pathophysiology of ALS and FTD. RIPK1 is a critical signaling protein that mediates inflammatory and cell death signaling. Loss of function mutations in *OPTN* and *TBK1* can lead to familial ALS/FTD and their deficiency causes hyperactivation of RIPK1 kinase activity. Genetic and pharmacological inactivation of RIPK1 rescues the increased RIPK1 activation and necroptosis in the spinal cords of $Optn^{-/-}$ mice. Additionally, TBK1 is a negative regulator of RIPK1 kinase activity by phosphorylation at T189 and the embryonic lethality of $Tbk1^{-/-}$ mice is rescued by genetic inactivation of RIPK1 kinase activity. Previous studies have suggested increased RIPK1 expression in $SOD1^{G93A}$ mouse models and ALS patient samples and while these data suggest activation of RIPK1 in the central nervous system (CNS) may mediate ALS disease progression, we sought to definitively elucidate the role of RIPK1 kinase activity in the $SOD1^{G93A}$ mouse model and in ALS patient-derived post-mortem samples. Using a mouse active CNS-penetrant RIPK1 inhibitor, we show that blocking RIPK1 kinase activity attenuates disease phenotypes in the SOD1 model of ALS and improves the age-dependent behavioral deficits in this mouse model. The role of RIPK1 kinase activity in this model is also assessed using a multi-omics approach. Furthermore, we demonstrate RIPK1 activity and expression is increased in sporadic ALS spinal cord and motor cortex samples relative to control. Based on our previous data we suggest that aberrant RIPK1 activation in various cell types in the CNS contributes to motor neuron loss in ALS.

Disclosures: F. Pontarelli: None. **M. Zelic:** None. **M. Lamorte:** None. **T. Treleaven:** None. **E. McGuirk:** None. **Y. Ren:** None. **X. Tang:** None. **F. Huang:** None. **J. Dodge:** None. **N. Atassi:** None. **T. Hammond:** None. **D. Ofengeim:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.07

Topic: C.06. Neuromuscular Diseases

Support: RISE Scholarship
NSF IOS1608147
NIH R15HD075207
NIH P12GM103653
NIH R25NS095371

Title: Spinal Muscular Atrophy (SMA) Differentially Reduces Cerebellar Structure and Axon Development in SMNΔ7 Mice: An MRI Study

Authors: *N. COTTAM, C. CHARVET, M. HARRINGTON, J. SUN;
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Abstract: Spinal Muscular Atrophy (SMA) is an autosomal recessive disease that affects 1 in every 6,000-10,000 individuals at birth, making it the leading genetic cause of infant mortality. There are effective treatments for SMA, but not all patients respond to them, and their long-term effects are unclear. SMA is best defined by motor neuron dysfunction due to a depletion of ubiquitous survival motor neuron protein (SMN) in motor neurons, leading to their degeneration at the neuromuscular junction in the anterior horn of the spinal cord. However, due to the key roles of SMN in cellular function, a growing number of studies support that SMA is a multi-system disease. Moreover, the spinal cord has received much attention; but the cerebellum hasn't, even when widespread pathology has been reported in the cerebellum of SMA patients. Due to its important role in motor function, we performed an exploratory study to investigate it's development using the SMNΔ7 mouse model. Mouse genotypes were confirmed using a mass measurement and righting test. We acquired volumetric brain data using T2-weighted images from magnetic resonance imaging scans (dMRI) and segmentation. We found that cerebellar growth is significantly reduced in SMA-affected mice compared to wild type. Furthermore, the cerebella of late-stage SMA (P12, post-natal day 12) mice are differentially affected relative to other brain regions, despite an observed decrease in whole-brain volume in SMA. To further define SMA disease pathology in the cerebellum, we studied the tractography using diffusion tensor imaging (DTI) to quantify white matter pathway abnormalities in SMA. Inferior peduncular tracts and intracerebellar connections were significantly diminished by the disease in the P12 ex-vivo SMNΔ7 mice brain scans relative to wild type. To better understand whether these cerebellar effects are downstream pathologies or play an important role in disease development, future work will consist of similar analyses on P3-P4 mice brain scans, immunohistochemistry, and acquiring DTI metrics such as fractional anisotropy, radial, medial, and axial diffusivity at P3-P4 and P12. We conclude that cerebellar axonal development and concomitant white matter pathways are affected by SMA. A better basic understanding of these neurobiological abnormalities of SMA may lead to novel and more effective avenues for treatment.

Disclosures: N. Cottam: None. **C. Charvet:** None. **M. Harrington:** None. **J. Sun:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.08

Topic: C.06. Neuromuscular Diseases

Support: NIH RO1 Grant NS078375-06
AFM-Téléthon

Title: Serotonergic neuromodulation is impaired in a mouse model of spinal muscular atrophy

Authors: *N. DELESTRÉE, E. SEMIZOGLOU, J. G. PAGIAZITIS, A. VUKOJICIC, E. DROBAC, V. PAUSHKIN, G. Z. MENTIS;
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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease due to deficiency of SMN protein and is characterized by motor neuron loss, muscle atrophy and impairment of motor function. Gait, posture and locomotion, which are largely influenced by both proprioception and neuromodulation, are altered in SMA patients. We previously reported that one of the earliest hallmark of the disease is the dysfunction and elimination of proprioceptive synapses mediated by the classical complement pathway. However, whether the neuromodulatory serotonergic system is affected in SMA, is not known.
To address this, we utilized the SMN-Δ7 mouse model of SMA and investigated the morphological and functional characteristics of the serotonergic (5-HT) synapses on spinal motor neurons. We found that 5-HT synaptic coverage is reduced by 25% as early as P4 in vulnerable (L1 and L5-MMC) but not resistant (L5-LMC) motor neurons in SMA and that this reduction progressed to ~55% in vulnerable motor pools of P10 SMA animals. We also found that the proportion of 5-HT synapses tagged by the complement protein C1q is increased in SMA mice, suggesting that the complement pathway is involved in 5-HT synapses pruning in SMA. Functionally, 5-HT is known to modulate the monosynaptic sensory-motor reflex mediated by proprioceptive synapses on motor neurons. Using an *ex vivo* brainstem-spinal cord preparation, we confirmed that stimulation of the brainstem reduces the amplitude of this reflex by 50% in P10 WT animals. However, while this modulatory effect was preserved in SMA animals in the resistant L5 segment, it was reduced by 90% in the vulnerable L1 spinal segment. Selective restoration of SMN in 5-HT neurons using a conditional inversion SMA mouse line, in which Cre is expressed under the *Pet1* promoter, resulted in restoration of 5-HT synapses in the vulnerable motor pools (~90% of WT levels), and restored the 5-HT neuromodulation effect on the L1 spinal reflex compared to their SMA counterparts. Behaviorally, the dysfunction of 5-HT synapses in SMA resulted in impairment of locomotor behavior as observed from EMG activity from TA muscles in freely moving mice at P10. In contrast, selective restoration of SMN in 5-HT neurons improved significantly left-right hindlimb muscle alternation during locomotor activity. Together, our study uncovers a severe serotonergic dysfunction in SMA mice and proposes the serotonergic pathway as a new therapeutic target.

Disclosures: N. Delestrée: None. **E. Semizoglou:** None. **J.G. Pagiazitis:** None. **A. Vukojicic:** None. **E. Drobac:** None. **V. Paushkin:** None. **G.Z. Mentis:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.09

Topic: C.06. Neuromuscular Diseases

Support: NIH R01 NS081303

Title: Ddx17 regulates fus-mediated neurodegeneration in an rgg-domain dependent manner

Authors: T. FORTUNA[1], S. KOUR[2], E. N. ANDERSON[2], C. WARD[2], D. RAJASUNDARAM[2], C. J. DONNELLY[3], H. WYNE[4], F. SHEWMAKER[5], ***U. PANDEY**[1]; [2]Pediatrics, [1]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [3]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [4]Biochem., Uniformed Services Univ., Bethesda,, MD; [5]Biochem., Uniformed Services Univ., Bethesda, MD

Abstract: Mutations in Fused in Sarcoma (FUS) have been linked with juvenile and aggressive form of amyotrophic lateral sclerosis (ALS). Cytoplasmic aggregation and defects in DNA repair pathway have been linked with ALS pathogenesis. However, the molecular mechanisms and genetic modifers of DNA damage repair pathway are yet not known. While doing RNA-sequencing analysis of the *Drosophila* brains expressing FUS, we identified significantly altered genes and pathways involved in FUS-mediated neurodegeneration *in vivo*. We found that the DEAD-Box Helicase 17 (DDX17) expression level was significantly downregulated in response to mutant FUS in *Drosophila* and human cell lines. Interestingly, mutant FUS recruits nuclear DDX17 into cytoplasmic stress granules and biochemically interacts with DDX17 through the RGG1 domain of FUS. Importantly, genetic upregulation of DDX17 reduces cytoplasmic mislocalization and sequestration of mutant FUS into cytoplasmic stress granules. We identified DDX17 as a novel regulator of the DNA damage response pathway whose upregulation repairs defective DNA damage repair machinery caused by mutant neuronal FUS ALS. In addition, we show DDX17 is a novel modifier of FUS-mediated neurodegeneration in vivo. Our findings indicate DDX17 is downregulated in response to mutant FUS, and restoration of DDX17 levels suppresses FUS-mediated neuropathogenesis and toxicity in vivo

Disclosures: T. Fortuna: None. **S. Kour:** None. **E.N. Anderson:** None. **C. Ward:** None. **D. Rajasundaram:** None. **C.J. Donnelly:** None. **H. Wyne:** None. **F. Shewmaker:** None. **U. Pandey:** None.

Poster

P292. Motor Neuron Disease: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P292.10

Topic: C.06. Neuromuscular Diseases

Support: ALS Canada

Title: C-boutons and their Influence on Amyotrophic Lateral Sclerosis Disease Progression

Authors: *T. L. WELLS, J. R. MYLES, T. AKAY;
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease with progressive motor neuron death, where patients usually die within five years of diagnosis. Previously we showed that the C-boutons, which are large cholinergic synapses to motor neurons that modulate motor neuron activity, are necessary for behavioural compensation in mSOD1^{G93A} mice, a mouse model for ALS. Since the C-boutons likely increase the excitability of surviving motor neurons to compensate for motor neuron loss during ALS disease progression, we reasoned that amplitude modulation through the C-boutons likely increases motor neuron stress and worsens disease progression. By comparing male and female mSOD1^{G93A} mice to mSOD1^{G93A} mice with genetically silenced C-boutons (mSOD1^{G93A}; Dbx1::cre; ChAT$^{fl/fl}$) (mSOD1^{G93A}/C^{off}), we show that the C-boutons do not influence the humane endpoint of mSOD1^{G93A} mice (mSOD1^{G93A} = 156.4 ± 7.3 days, n = 17; mSOD1^{G93A}/C^{off} = 152.5 ± 7.9, n = 12); however, histological assessment of the neuromuscular junction shows that C-bouton silencing significantly improves fast-twitch leg muscle innervation over time (mSOD1^{G93A} n = 18; mSOD1^{G93A}/C^{off} n = 11). Using behavioural analysis, we also provide evidence that C-bouton silencing in combination with swimming (mSOD1^{G93A}/C^{off} swim) is beneficial for the behavioral capabilities of mSOD1^{G93A} mice (mSOD1^{G93A} rest n = 5; mSOD1^{G93A} swim n = 5; mSOD1^{G93A}/C^{off} swim n = 11). Lastly, we provide evidence that C-bouton silencing in combination with swimming in mSOD1^{G93A} mice significantly delays the humane endpoint relative to C-bouton silencing alone, but not relative to mSOD1^{G93A} mice with C-boutons intact (mSOD1^{G93A}/C^{off} swim = 162.6 ± 6.8 days, n = 5, ANOVA $p<0.05$). Our observations suggest that manipulating the C-boutons in combination with a modulatory-targeted training program may therefore be beneficial for ALS patients.

Disclosures: T.L. Wells: None. **J.R. Myles:** None. **T. Akay:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.01

Topic: D.03. Somatosensation – Pain

Support: VA I21BX002471
VA I01RX001776
DoD GW190045

Title: Chronic pain and fatigue symptoms in a mouse model of Gulf War Illness

Authors: H. NGUYEN[1], P. SAHBAIE[2], L. GOBA[4], J. SUL[4], A. SUZAKI[4], D. J. CLARK[2], ***T.-T. HUANG**[3];
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Abstract: More than twenty five years after the end of the Gulf War (1900-1991), a substantial contingent of veterans still suffer from a number of health problems affecting the neurological and musculoskeletal systems; the most common symptoms include chronic pain and fatigue. This constellation of symptoms is collectively termed Gulf War Illness (GWI). The etiology of GWI remains elusive, yet likely involves exposure to various chemicals as well as physical and psychological stresses unique to the GW theatre. Using a mouse model of GWI, by repeatedly exposing mice to a combination of Gulf War chemicals (pyridostigmine bromide, permethrin, N,N-Diethyl-m-toluamide, and chlorpyrifos) and mild immobilization stress, we assessed their pain susceptibilities and fatigue symptoms at various intervals following Gulf War agents exposure. The mouse model recapitulated several aspects of the human illness, including hyperalgesia, impaired descending inhibition of pain, and increased tonic pain. There is a close association between chronic pain and fatigue in GWI patients. Consistent with this clinical observation, the mouse model showed a significant reduction in physical endurance on the treadmill. Examination of skeletal muscles suggested that reduction in mitochondrial functions may be the source of the fatigue symptoms. Increased oxidative stress has been implicated in GW agent exposure. To assess whether enhanced antioxidant capacity can counter the effects of GW agents, transgenic mice overexpressing extracellular superoxide dismutase (SOD3OE) were examined in this study. The negative impacts of GW agents, particularly in pain susceptibilities, were largely diminished in SOD3OE mice, suggesting that increased oxidative stress was associated with the development of these Gulf War symptoms.

Disclosures: H. Nguyen: None. **P. Sahbaie:** None. **L. Goba:** None. **J. Sul:** None. **A. Suzaki:** None. **D.J. Clark:** None. **T. Huang:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.02

Topic: D.03. Somatosensation – Pain

Support: PAPIIT-IN219720
CONACyT fellowship

Title: Sex differences in the behavioral changes of brief-access alcohol consumption induced by trigeminal nerve injury in C67BL/6 mice

Authors: *R. ANDRADE GONZALEZ[1,2], E. PERRUSQUIA HERNANDEZ[1,2], M. HERNANDEZ CAMPOS[1], I. PEREZ MARTINEZ[2];
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Abstract: Chronic pain is a significant risk factor for developing alcohol use disorder in different rates between men and women. During regular alcohol consumption, a disruption of goal-directed control promotes the transition from casual to chronic alcohol consumption and increased ethanol consummatory behaviors; the former is predictive of alcohol use disorder development. Prior work has shown that chronic pain promotes alcohol consumption exclusively in male rodent models. However, the underlying processes that control this increased consummatory behavior haven't been elucidated. This work aimed to assess the behavioral changes in male and female C57BL/6 mice alcohol consumption patterns with trigeminal neuropathic nerve injury. Following mental nerve injury or sham surgery in male and female mice, behavioral tests were performed to evaluate sensory impairments and changes in the licking microstructure of alcohol consumption in a brief access test. Our results showed that mice with mental nerve injury developed allodynia, hyperalgesia, and spontaneous pain, more severely in male rodents, observed as hypersensibility to mechanical stimulation and excessive facial grooming. Female mice in both groups showed an increased alcohol consumption compared to males, related to more licks in a cluster. Mental nerve injury caused a more efficient licking behavior in male mice, as quantified by the Mean Primary ILI. Our data demonstrate that female mice consume alcohol and quinine in an aversion-resistant manner, regardless of the treatment. In contrast, male mice with mental nerve injury are susceptible to higher alcohol consumption in prolonged access periods due to greater automaticity in alcohol drinking.

Disclosures: R. Andrade Gonzalez: None. **E. Perrusquia Hernandez:** None. **M. Hernandez Campos:** None. **I. Perez Martinez:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.03

Topic: D.03. Somatosensation – Pain

Title: Thermal nociception in large Theraphosid spiders using the plantar Hargreaves test apparatus

Authors: A. UPTON[1], B. KENNEDY[2], *D. COHN[3], C. TAYLOR[3], S. BAKER[4], C. TRIM[1], **S. TRIM**[4];
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Sandwich, United Kingdom; [3]Transpharmation Ltd, Sandwich, United Kingdom; [4]Venomtech Ltd, Sandwich, United Kingdom

Abstract: Where model invertebrates have been used for nociception, they have typically been selected for their rapid generation time and potential for genetic manipulation. This has often left challenges with regards to behaviour assessment and pharmacological interventions. To date, arachnid pain research has been focused on true spiders (Aranaeomorphae) such as the wandering spider *Cupiennius* salei and jumping spiders *Servaea incana* with only a preliminary mention of theraphosidae. Theraphosid spiders, erroneously called tarantulas, are large (several over 25cm and 100g) hirsute arachnids commonly kept in zoological collections, as private pets and increasingly laboratories thus making them available for further study. In contrast to flies and nematodes, theraphosids are of similar size to small rodents and thus suitable for use in experimental equipment designed for rodents, with some caveats and extra considerations. Although spiders are not protected under ASPA all work plans had passed through ethical review. To understand if and how theraphosids respond to thermal laser stimulation 39 animals representing eight Genera were tested after a period of acclimatisation to the test chamber, similar to the rodent protocol. Thermal nociception testing using the standard rodent Plantar Hargreaves test apparatus (Ugo Basile, Gemonio, Italy) demonstrated that the instrument was sensitive enough to detect the theraphosid tarsus (aka foot) and its subsequent withdrawal, recording the time as expected. Each spider was tested three times with three different legs. One of the benefits of spiders is that they still have five more untested legs. This baseline study demonstrated that Theraphosid spiders respond to a thermal stimulation with a foot withdrawal in a similar way to rodents and many individuals exhibited foot rubbing after stimulation. Withdrawal latencies means were between 10 and 18 seconds and varied between Genera and with weight of animal. Spiders of the Genus of *Grammostola* were the least variable and therefore used for the follow-on studies of sensitisation. There was a slight trend ($R^2 = 0.38$) towards heavier spiders having longer latencies, but as it is normal practice to age and weight match study animals, so this was not unexpected. Using the *Grammostola* spp. Spiders further demonstrated a significant ($P = 0.007$) reduction in withdrawal latency to a repeated stimulation. A needle stick injury in the plantar surface of the tarsus generated a 35% reduction in withdrawal latency which took 52 days to return to normal levels. This is the first study to show utility of the Hargreaves equipment in invertebrates.

Disclosures: A. Upton: None. **B. Kennedy:** None. **D. Cohn:** None. **C. Taylor:** None. **S. Baker:** None. **C. Trim:** None. **S. Trim:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.04

Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award #ADHS18-198875
University of Arizona - institutional funds

Title: Systematic Isoform-Selective Heat Shock Protein 90 Inhibitors Improve the Therapeutic Index of Morphine

Authors: *K. CHOU, C. S. CAMPBELL, P. BENJARANO, D. DURON, J. M. STREICHER; Univ. of Arizona, Tucson, AZ

Abstract: Opioids are the benchmark for severe acute pain; however, severe side effects including respiratory depression and addiction limit their viability. One approach to improve opioid therapy focuses on a dose-reduction strategy by amplifying opioid analgesia without boosting side effects. To this end, we've focused on Heat shock protein 90 (Hsp90), a central protein regulator in the cell; ongoing studies have shown that inhibition of Hsp90 in the spinal cord improves morphine antinociceptive potency by 2-4 fold in acute and chronic pain while reducing tolerance, rescuing established tolerance, and not changing reward and constipation, enabling a dose-reduction strategy. However, our results also showed that non-selective Hsp90 inhibitors given systemically (intravenous, IV) have the opposite effect, blocking opioid pain relief. To surmount this roadblock, we determined which Hsp90 isoforms regulate opioid pain relief in brain and spinal cord in mice using selective small molecule inhibitors and CRISPR/Cas9 gene editing. We found that Hsp90α alone regulated opioid signaling and pain relief in the brain. However, in the spinal cord, we found that the isoforms Hsp90α, Hsp90β, and Grp94 all regulates opioid signaling and pain relief. This led to our hypothesis that targeting spinal cord Hsp90 with isoform-selective inhibitors given IV could boost opioid pain relief without altering side effects, enabling a dose-reduction strategy. We tested this hypothesis with the novel Grp94-selective inhibitor KUNG65 and the novel Hsp90β-selective inhibitor KUNB106, given at a 1mg/kg dose IV in male and female CD-1 mice, followed by a 24hr treatment time, then analysis of opioid antinociception and side effects. We found that systemic (IV) KUNG65 treatment resulted in a 1.9 fold increase in morphine potency to relieve tail flick pain with similar findings for KUNB106, consistent with our earlier studies injecting inhibitors directly into the spinal cord. We also found that both KUNG65 and KUNB106 boosted morphine potency in paw incision pain. Additionally, we found that KUNG65 and KUNB106 injection could rescue established morphine tolerance in the tail flick assay, again as we found for direct spinal cord injection. These results support our hypothesis that isoform-selective Hsp90 inhibitors can engage Hsp90 in the spinal cord when given systemically, resulting in improved opioid antinociception and side effects; strongly suggesting that Hsp90 isoform-selective inhibitors could be a powerful new tool to improve opioid therapy through a dose-reduction strategy, and further show that this effect can be achieved through a translationally relevant dosing route.

Disclosures: K. Chou: None. **C.S. Campbell:** None. **P. Benjarano:** None. **D. Duron:** None. **J.M. Streicher:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.05

Topic: D.03. Somatosensation – Pain

Support: NIGMS 5U54GM104942-03
NCCIH 1R21AT011238 (RJN)

Title: Dim light at night differentially alters mechanical sensitivity in female and male TALLYHO/JngJ mice

Authors: *R. C. WHITE, J. R. BUMGARNER, J. A. LIU, R. J. NELSON;
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Abstract: Circadian rhythms are endogenous physiological and behavioral cycles that have a period of about 24-hours. These rhythms are synchronized to the environment by several entraining cues, the primary of which is light early during the day. Disrupted circadian rhythms are caused by irregular synchronizing cues, for example, exposure to dim light at night (dLAN). The induction of artificial LAN in society has become ubiquitous, affecting 80% of the global human population. LAN has adverse effects on several physiological functions, such as metabolism, locomotor activity, clock genes, peripheral and central inflammation, obesity, and weight gain. Importantly, dLAN exposure has been associated with exacerbated pain behavior in rodents, specifically cold hyperalgesia and mechanical hypersensitivity. Type ll diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance that affects millions of individuals every year. Diabetic peripheral neuropathy is a common side effect of T2DM, impacting more than 50% of patients. In this study, we sought to examine the effects of dLAN exposure (5 lux) on the progression of diabetic peripheral neuropathy in TALLYHO/JngJ (TH) mice that develop T2DM. We hypothesized that dLAN exposure exaggerates pain behaviors associated with the early stages of diabetic neuropathy. We observed that 4-weeks of dLAN exposure differentially altered mechanical withdrawal thresholds in male and female TH mice, leading to hypo- and hyperesthesia, respectively. These data suggest that dLAN exposure may play a role in the progression of hyper- or hypo-esthesia associated with T2DM.

Disclosures: R.C. White: None. **J.R. Bumgarner:** None. **J.A. Liu:** None. **R.J. Nelson:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.06

Topic: D.03. Somatosensation – Pain

Support: CIHR FRN154281

Title: Sex differences in the long-term course of neuropathic pain behavior after spared nerve injury

Authors: M. MILLECAMPS[1], ***L. CHAUDHRY**[2], S. SOTOCINAL[2], L. S. STONE[3,2], J. S. MOGIL[2];
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Abstract: The vast majority of preclinical pain literature uses only young male rodents as subjects, and contains experiments lasting only several weeks after the painful injury. This *status quo* has a major disconnect with the clinical reality: chronic pain patients who tend to be older, female, and—by definition—with pain lasting more than 3 months. To study long-term trends in pain behavior and lifespan, we repeatedly tested male and female C57BL/6 mice over the course of 24+ months following a spared nerve injury (SNI). Early after surgery, both sexes display equivalent levels of mechanical hypersensitivity, measured via von Frey testing. As we have previously observed, hypersensitivity resolved by 6 months post-SNI in female mice, while remaining at high levels in male mice. Unexpectedly, starting at 12 months post-SNI, mechanical hypersensitivity reappears in female mice, at levels equaling or even exceeding that of males. This parabolic pattern in female mice is also observed for cold pain (acetone drop test) and guarding behavior (Attal scores). The impacts of chronic pain on lifespan are currently inconclusive, as most mice in both SNI and sham surgery groups remain alive. Ongoing experiments are investigating underlying mechanisms for these sex-specific patterns of behavior, with one possible explanation involving the development of pain-maintaining microglial senescence at different time points post-injury in male versus female mice. We believe that preclinical pain researchers who wish to study truly “chronic” pain must incorporate much longer time points after injury.

Disclosures: M. Millecamps: None. **L. Chaudhry:** None. **S. Sotocinal:** None. **L.S. Stone:** None. **J.S. Mogil:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01AT006978

Title: Effects of Spinal Manipulation and Mobilization techniques on trunk compliance in chronic low back pain: A secondary analysis of the RELIEF Study

Authors: *A. STAMENKOVIC[1], R. SABO[1], S. M. VAN DER VEEN[1], B. C. CLARK[2], P. E. PIDCOE[1], J. S. THOMAS[1];
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Abstract: Mechanisms of chronic pain are multifaceted and can be driven by biological, psychological and social factors. For low back pain, therapeutic interventions such as spinal manipulation and mobilization are sometimes considered first-line treatment options based on their potential to reduce internal mechanical stresses, and influence para-spinal primary afferent activity in the propagation of central sensitization. As these chronic pain mechanisms are associated with increases in low back stiffness (or decreases in trunk compliance), we examined whether such therapeutic interventions influenced a simple measure developed by the authors to quantify trunk compliance.

The RELIEF Study was a RCT comprised of a 3-week intervention, and 4-week follow-up to examine the effectiveness of thrust-based spinal manipulation, to non-thrust spinal mobilization, and a sham cold laser placebo. A sample of the cohort (n=58, 57% female) underwent seated trunk-based perturbations using a custom-designed multi-axial perturbation device, at baseline, immediately following the first intervention session, upon the completion of all intervention sessions, and after 4 weeks. A trunk compliance index (TCI, defined as the inverse of stiffness) for each perturbation direction (eliciting trunk flexion, extension, left and right lateral flexion, and left and right rotation) was calculated by dividing the magnitude of trunk displacement due to the perturbation, by the force of the applied perturbation load normalized to participant body weight. We fit a linear mixed effects model (fixed effects: Treatment, Time, Treatment*Time) for each direction accounting for differences of trunk compliance at baseline, and including sex, and baseline disability and pain scores as covariates. Regardless of perturbation direction, there were no significant interactions between treatment groups across any of the recorded time points (e.g., flexion: Treatment*Time, $F_{(6,147.7)} = 0.37$; $p = 0.895$). These findings are consistent with the clinical outcomes from the RELIEF Study, which found no effect of intervention on measures of pain or disability.

Of note, when compared to a matched healthy control cohort, the TCI is shown to be significantly reduced in 5 of 6 directions in back pain individuals and has strong distinguishing capacity (ROC area under curve = 0.898; sensitivity = 93.88; specificity = 73.47). Our novel measure for trunk compliance (i.e., TCI) should be examined in future work to determine its robustness as a biomarker of low back pain.

Disclosures: A. Stamenkovic: None. **R. Sabo:** None. **S.M. Van Der Veen:** None. **B.C. Clark:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research

relationship even if those funds come to an institution.; NIH, Osteopathic Heritage Foundations, American Osteopathic Association. **P.E. Pidcoe:** None. **J.S. Thomas:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS099245
NIH Grant R01NS069568

Title: Nerve constriction with a synthetic absorbable suture does not model centralized chronic pain in rats

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Abstract: Chronic pain severely impacts quality of life. Understanding the mechanisms underlying chronic pain is a high priority due to the associated economic burden, inadequate treatment options, and clinical impact. Elucidating the transition from acute to chronic pain is especially vital; doing so requires the use of valid animal models. Chronic pain often persists in the absence of tissue damage. An informative animal model of chronic pain should replicate this finding. Many animal studies model neuropathic pain by constricting a peripheral nerve with an absorbable suture or removable cuff. These studies assume that pain persists after removing the constriction, modeling chronic pain in humans. Data from cuff constriction models suggest that, while reflexive signs of pain resolve when the cuff is removed, affective components of pain persist (PMID: 25209272). Here, we modeled trigeminal neuropathic pain using a vicryl ligature-based chronic constriction injury of the infraorbital nerve (CCI-ION) in 7 male and 12 female Wistar rats. We sought to determine whether reflexive and ongoing signs of pain (assessed by facial tactile sensitivity and facial grimace scoring, respectively) persist in the absence of nerve pathology. At 2 weeks and 4 weeks post CCI-ION, tactile thresholds were lower than baseline, suggesting tactile hypersensitivity (2 weeks: Wilcoxon matched pairs test, W= -143.0, p=0.0008, Cohen's d=0.94, large effect size. 4 weeks: Wilcoxon matched pairs test, W=-148.0, p=0.0017, Cohen's d=0.82, large effect size). Grimace scores at these time points were higher than baseline, indicating ongoing pain at 2-4 weeks post-CCI (2 weeks: paired t-test, t(18)=5.960, p<0.0001, Cohen's d=1.72, large effect size. 4 weeks: Wilcoxon matched pairs test, W=186.0, p<0.0001,

Cohen's d=1.58, large effect size). By 8 weeks post-CCI, tactile thresholds were indistinguishable from baseline values (Wilcoxon matched pairs test, W=-5.0, p=0.90), whereas grimace scores remained elevated ($t(14) = 3.749$, p=0.0022, Cohen's d=1.22, large effect size). By 11 weeks post-CCI, grimace scores returned to baseline values (paired t-test, $t(7)=0.92$, p=0.39). Despite resolution of pain behaviors to baseline levels, 7 out of 8 infraorbital nerves, harvested at 15 weeks post-CCI, had histology findings of ongoing inflammation or foreign body reaction in the injured nerve. Thus, the vicryl-based CCI-ION model does not represent the development of purely central chronic, neuropathic pain.

Disclosures: O. Uddin: None. **R. Masri:** None. **A. Keller:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.09

Topic: D.03. Somatosensation – Pain

Support: Alberta Innovates mCannabis Grant G2020000213

Title: The analgesic effects of cannabinoids and terpenoids on a Zebrafish model for nociception and pain

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Abstract: With many communities affected by the opioid crisis, it has become of paramount importance to explore methods of pain management outside of treatments with opioids. One alternative that has seen a recent surge in interest is the cannabis- and related phytochemical-based treatments. Some cannabinoids and terpenoids have been shown to have analgesic and anxiolytic effects in various model systems; however, the analgesic effects of the vast majority of terpenoids in cannabis plants remain unknown. In this study I used a zebrafish model of nociception and pain to test the analgesic effects of cannabinoids, terpenoids and a combination of these compounds. Zebrafish provides a robust and high-throughput model system for such inquiry and may serve as a more ethical alternative compared to other conventional models of pain. We used acute exposure to acetic acid as a noxious stimulus, which provides a means by which to assess nociception and analgesia. Using behavioural assays, we confirmed the ability of acetic acid to elicit a change in locomotion in larval zebrafish. Acetic acid at 0.1% resulted in a decrease in the mean activity of 5 day old (dpf) zebrafish compared to controls. Exposure to low concentrations of acetic acid (0.001% - 0.01%), on the other hand, led to a drastic increase in mean activity in the larvae – which was approximately four times the activity seen in the controls. An acute treatment with cannabidiol (CBD) at 2.5 µg/mL, 5 µg/mL and 10 µg/mL,

prevented the subsequent acetic acid-induced increase in movement. Interestingly, tetrahydrocannabinol (THC) and certain terpenoids also found in cannabis, failed to prevent the effects of acetic acid by themselves but showed some ability to enhance the preventative effects of CBD when administered in conjunction with it. Together, CBD at 2.5 µg/mL and THC at concentrations as low as 0.5 µg/mL showed activity levels that were not significantly different from that of the baseline control. Similar results were observed with terpenoids such as α-pinene at 50 µg/mL and linalool at 1 µg/mL. These findings lay the foundation for a more thorough investigation of the therapeutic effects of cannabinoids, terpenoids and combinations of the two, as well as a mechanistical understanding of how such effects arise at cellular and systemic levels.

Disclosures: A. Kim: None. **W. Allison:** None. **D. Ali:** None.

Poster

P393. Pain Models: Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P393.10

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Effect of ceftriaxone on nociceptive behaviors induced by spinal nerve ligation

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Abstract: "Effect of ceftriaxone on nociceptive behaviors induced by spinal nerve ligation" Introduction: the International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or resembling such damage". According to its neurophysiological mechanism, pain can be classified as nociceptive, inflammatory, dysfunctional, and neuropathic. The lASP defines neuropathic pain (NP) as "pain caused by injury or disease of the somatosensory nervous system" and can be classified according to its anatomical location as central or peripheral. Studies suggested that beta-lactam drugs are involved in neuropathic pain, the administration of beta-lactam drugs, such as ceftriaxone, have shown antiallodynic, antihyperalgesic, and antinociceptive effects on inflammatory pain. Objective: the aim of this study was to evaluate the effect of ceftriaxone on nociceptive behaviors induced by spinal nerve ligation (SNL) in Wistar strain rats. Material and methods: experimental study, 36 female Wistar strain rats of 140-160 g were used and divided into the following groups: naive, sham, SNL rats treated with saline solution, SLN rats treated with pregabalin 30 mg/kg p.o., SNL rats treated with gabapentin 300 mg/kg p.o., SNL rats treated with ceftriaxone at doses of 10 and 100 mg/kg intraperitoneally (i.p.). Nociceptive behaviors were determined by the up and down method. This experiment was

performed according to international guidelines on ethical aspects for experimental pain research in animals. Results: in the establishment of the allodynic behavior induced by spinal nerve ligation, a reduction of the paw withdrawal threshold was observed in the time course, which occurred from day 1 and was maintained for 28 days post-ligation. With the administration of pregabalin and gabapentin, antiallodynic behavior was observed in the time course and the areas under the curve (AUC) of the time course of antiallodynic behavior, a significant difference was observed between the pregabalin, and gabapentin groups compared to vehicle with a value of p=0.0001. The administration of ceftriaxone at a dose of 100 mg/kg i.p., showed an antiallodynic response starting at 30 minutes and reached its maximum peak at two hours in the time course, and in the AUC a significantly different response was observed from the vehicle group with a value of p=0.0001. Conclusions: Ceftriaxone was shown to induce an antinociceptive effect in rats subjected to SNL, and a dose-dependent behavior of ceftriaxone (10-100 mg/kg i.p.) was observed.

Disclosures: C.G. Guzman Priego: None. **I.I. Ramos Rodriguez:** None. **E. Rodríguez Palma:** None. **J.E. Torres Lopez:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.01

Topic: D.04. Somatosensation – Touch

Support: R01 NS117597
R01 HD054453

Title: Mechanisms of sensory adaptation over behaviorally relevant time scales in mouse barrel cortex

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Abstract: Typical nervous system function depends on the ability of sensory circuits to adapt their activity to the statistics of the surrounding environment and filter out irrelevant stimuli (e.g., non-salient or non-threatening). Stimulus-specific sensory adaptation, characterized by a progressive decrease in neuronal activity with ongoing stimulation, is a universal phenomenon across species and sensory modalities. Most previous studies of adaptation focused on rapid timescales (less than 500 ms), but much less is known about adaptation to sensory stimuli occurring over tens of seconds, which is more relevant to an animal's behavior. Here, we assess

the role of adaptation in the barrel field of the mouse somatosensory cortex (S1BF), a crucial region for processing tactile stimuli transmitted through the whiskers. Whether such adaptation is stimulus-specific, and whether S1BF neurons exhibit the same adaptive firing patterns across a variety of stimuli has not been explored. We investigated adaptation within S1BF to an array of stimuli of various durations and intervals and asked if it can generalize across stimuli of different frequencies. We performed in vivo 2-photon calcium imaging (GCaMP6s) of layer 2/3 excitatory neurons in awake Slc17-Cre x Ai162 mice during repetitive whisker stimulation. To assess whether adaptation is comparable across different stimuli, we exposed mice to bouts of 10 repetitive whisker stimulations at various stimulus durations and interstimulus intervals. S1BF neurons exhibited dynamic and complex response profiles, with some cells exhibiting classic adaptive responses and a minority showing facilitating responses; still, most responsive neurons fired inconsistently to individual stimuli with fluctuating response magnitudes. To assess whether adaptation generalizes, we performed frequency switch experiments, exposing mice to a bout of 10 repetitive whisker stimulations followed by a second bout at an alternate frequency. Switching to a higher alternate frequency attenuated adaptation in strongly adapting neurons, while switching to a lower one did not. Future studies will explore the mechanisms of sensory adaptation over these prolonged time scales to better understand how the neuronal representation of sensory stimuli changes with repetitive exposure.

Disclosures: Z. Dobler: None. **C. Portera-Cailliau:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.02

Topic: D.04. Somatosensation – Touch

Support: NIH Grant R01NS109965
DP2NS111134

Title: Automated home-cage mouse training of a whisker-based tactile working memory task

Authors: *D. LEE[1,2], G. HOUSE[3], A. CAREY[3,2], D. LAMAY[3,4], J. L. CHEN[3,1,2];
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Abstract: Understanding the neural correlates of task learning requires unbiased observation of learning behavior. Human-guided mouse training of complex goal-directed tasks is a time-consuming process. This method of training limits the number of animals which can be trained in a given period of time and introduces potential human error and biases in the training of subjects. To increase training throughput and assess variability in learning in an unbiased manner, we

have designed an automated training system which allows mice to learn a whisker-based tactile working memory task from their home cage. The system contains components to deliver whisker and auditory stimuli, deliver rewarding and aversive stimuli, and monitor animal entry and licking behavior. Multiple animal cages can be connected to a shared training system. Singly housed mice gain scheduled access to the training module in a manner that enables simultaneous training of multiple animals while controlling for social interactions and hierarchies. We have developed software that intelligently tracks mouse performance and adjusts training settings across multiple stages of training on a two-alternative forced choice delayed match to sample task. Mice can successfully be trained to expert performance within three to four weeks. The learning trajectory of individual animals can be analyzed using a dynamic model of sensory decision-making fit to behavioral data. Through this, variability in learning and behavior strategies are observed across both inbred and outbred mouse strains.

Disclosures: D. Lee: None. **G. House:** None. **A. Carey:** None. **D. LaMay:** None. **J.L. Chen:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.03

Topic: D.04. Somatosensation – Touch

Title: Cortical processing dynamics during sensory-guided behavior using a tactile virtual-reality task

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Abstract: Our goal is to determine how neuronal populations in the mice barrel cortex are organized in dynamic functional networks that guide behavioral choices downstream during a whisker-dependent task. To enrich the number of activity states that neuronal populations encounter during a task trial, we designed naturalistic tactile virtual reality [1] behavioral experiments that load cortical circuits with difficult yet manageable cognitive tasks and, at the same time, elicit an ethologically relevant behavior. The head-fixed animal runs freely on a suspended ball treadmill with movement tracked in a 2-dimensional VR plane. Moveable walls on either side of the animal snout are coupled to the animal run, thus producing an illusion that the animal is running in a winding virtual corridor with programmed left and right turns being guided only by the whiskers touching the walls. Of specific interest for our study is the choice period: the time interval when the animal first senses the approaching wall with whiskers until it makes a choice to change the direction of his run. To record from large neuronal populations

during active navigation in a tactile VR, 64channel multi-electrode arrays are acutely implanted into the principle whisker barrel thus recording massive single-unit spiking activity across all cortical layers. Using Scnn1a-TG3-Cre x Ai32 mice, with Cre expression limited to 85% excitatory neurons in layer 4 of the cortex, enables optical tagging of individual L4 stellate cells as well as to manipulate these populations using optogenetics. We observed distinct layer-dependent stratification of spiking activity of neuronal populations recorded simultaneously during naturalistic whisker-dependent behavior in our VR. To uncover latent temporal dynamics of cellular-level interactions in recorded neuronal populations during the choice period we focused on analysis of pair-wise temporal correlations of spike timing. Both classical jitter-corrected cross-correlograms (CCG) and novel machine learning based analytical approaches reveal distinct dynamics of organization of neuronal populations into functional networks across all layers. The extracted patterns of dynamic connectivity are changing rapidly during the choice period at specific times for stimulus presentation, motor action preparation and choice execution when the running direction is changed. Observed correlation of whisker-guided behavior with dynamics of laminar network connectivity can help to reveal specific dynamic organization of columnar microcircuits and shed light on tactile information flow during perceptual decision making. [1] N.Sofroniew, et al, eLife;4:e12559 (2015)

Disclosures: A. Armstrong: None. **K. Hu:** None. **Y. Vlasov:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.04

Topic: D.04. Somatosensation – Touch

Support: NIH Intramural Research Program

Title: Brain-wide networks for functionally-distinct principal neuron in the primary somatosensory cortex

Authors: *A. R. INACIO, K. LAM, F. PEREIRA, C. R. GERFEN, S. LEE;
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Abstract: Principal neurons in the primary sensory cortices exhibit heterogeneous patterns of activity, not only in response to sensory stimuli but also, during spontaneous movements. Yet, how these heterogeneous, behavioral state-dependent patterns of activity arise is largely unknown. Longitudinal two-photon calcium imaging uncovered a remarkably stable correlation between the activity of principal neurons in layers II/III of the primary somatosensory cortex and spontaneous movements. This population activity could reliably predict spontaneous movements, but with a subset of neurons accounting for most of the prediction accuracy. The activity of

movement-encoding neurons could not be explained by direct sensory feedback, as paralysis of the contralateral whisker pad did not disrupt the correlation with ipsilateral whisker movements and locomotion. During pharmacological blockade of neuromodulators, the activity levels were altered, but the correlation of neuronal activity with spontaneous movements was largely maintained. By contrast, glutamatergic transmission blockers nearly abolished this correlation. Single cell-initiated monosynaptic retrograde tracing and whole-brain presynaptic network analysis revealed that movement-encoding neurons receive characteristic subcortical inputs. Finally, simultaneous imaging of layers II/III principal neurons and closed-loop optogenetic silencing of identified inputs during spontaneous movements altered spontaneous activity. Our study provides a connectivity rule that supports the representation of spontaneous movements in the primary somatosensory cortex.

Disclosures: A.R. Inacio: None. **K. Lam:** None. **F. Pereira:** None. **C.R. Gerfen:** None. **S. Lee:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.05

Topic: D.04. Somatosensation – Touch

Title: Stochastic resonance in the mouse barrel cortex by multisensory integration

Authors: *J. A. MARTINEZ CABRAL[1], P. E. TRUJILLO GARIBAY[1], L. P. OSUNA CARRASCO[1], C. TORO-CASTILLO[1], M. TREVINO VILLEGAS[2], B. *DE LA TORRE-VALDOVINOS[1];
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Abstract: Stochastic Resonance (SR) is a phenomenon that occurs in non-linear systems, where their response to a subthreshold signal is optimized by noise. Its presence has been demonstrated in different biological systems, ranging from simple models (i.e. mice) to more complex models (i.e. humans), also, a variety of techniques have been explored to test the scope of this phenomenon. Since SR has been applied mostly in integrative electrophysiology models, it is in our interest to implement an application of this phenomenon at a functional level in the nervous system, for which reason this study is intended to investigate whether the SR phenomenon occurs through multisensory integration in the mouse Barrel Cortex (BC).
Vibrissae were stimulated by mechanical protraction to 22 adult mice of the C57BL black strain (weight: 23 ± 5 grams) through two input signals, a pulse stimulus and a white noise signal modulated at 8 standard deviation levels applied in two different protocols: ipsilateral and contralateral route. An electrode in BC and a reference electrode were implanted to perform an

average of 4 Evoked Field Potential (EFP) recordings, in which the difference in amplitude and signal-to-noise ratio (SNR) was evaluated at each noise level applied.
When applying the ipsilateral noise protocol, there is an increase in the amplitude of the potential with an intermediate level of noise with respect to the basal level and after this level a decrease is observed ($P < 0.01$), the same for the SNR. In the case of the contralateral noise protocol, although less noticeable, a similar behavior is observed ($P < 0.05$). These results confirm the presence of SR since this behavior is characteristic of this phenomenon, which suggests that the properties of the signals that are introduced in different pathways are coupled in some structure previous to BC (e.g. thalamus or superior colliculi) through multisensory integration and it results in the appearance of this phenomenon in a subsequent structure, specifically, in this case, BC.

Disclosures: J.A. Martinez Cabral: None. **P.E. Trujillo Garibay:** None. **L.P. Osuna Carrasco:** None. **C. Toro-Castillo:** None. **M. Trevino Villegas:** None. **B. *De la Torre-Valdovinos:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.06

Topic: D.04. Somatosensation – Touch

Support: Maryland MPowering the State
NIH/NIDA F31-DA051113
NIH/NIGMS R25-GM055036
NIH/NIMH F31-MH123066
NIH/NIGMS T32-GM008181
NIH/NINDS T32-NS063391
NIH/NIDA R01-DA038613

Title: Environmental enrichment restores the long-lasting sequelae of perinatal fentanyl exposure

Authors: *J. B. ALIPIO[1], L. M. RIGGS[2], C. HAGA[1], M. E. FOX[1], K. ARAKAWA[1], R. BALAJI[3], M. PLANK[1], N. P. CRAMER[1], M. LOBO[1], A. KELLER[1];
[1]Anat. & Neurobio., [2]Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Univ. of Maryland Col. Park, College Park, MD

Abstract: The use of synthetic opioids, like fentanyl, among pregnant women has increased substantially in the last decade, leading to an increase in the number of infants exposed to opioids perinatally. This exposure can result in neuropsychiatric abnormalities that persist to

adolescence and even adulthood. To better understand the molecular, behavioral, circuit, and network-level abnormalities that result from fentanyl use during pregnancy, we developed a preclinical model of perinatal fentanyl exposure. We found that adolescent male and female C57Bl/6 mice exposed to fentanyl during perinatal development have increased anxiety-like behavior and sensory maladaptation. Consistent with the role of the primary somatosensory cortex (S1) in sensory-related processing, fentanyl-exposed mice had a reduction in the probability of spontaneous and evoked glutamate release onto S1 layer V neurons, and an increase in these metrics in layers 2/3. Expression of long-term potentiation (LTP) in S1 layer 2/3 neurons was also impaired. *In vivo* electrocorticogram activity in awake behaving mice revealed suppressed ketamine-evoked cortical oscillations. Morphological analysis of S1 pyramidal neurons showed reduced dendritic complexity, length, and reduced soma size. Fentanyl-exposed mice had abnormal cortical mRNA expression of key receptors involved in synaptic transmission, plasticity, and neuronal growth and development, consistent with the electrophysiological and morphological findings. We asked if these long-lasting effects can be restored by a non-invasive intervention. We demonstrate that developmental exposure to environmental enrichment ameliorates many of fentanyl's deleterious behavioral effects, including hyperactivity, enhanced sensitivity to anxiogenic environments, and sensory maladaptation. The deficits in synaptic function and plasticity were also restored by environmental enrichment. Environmental enrichment also affected neurons in control mice, who showed reduced LTP and long-term depression (LTD), and higher frequency of miniature excitatory postsynaptic currents, compared to mice raised in standard caging. These results demonstrate that the lasting somatosensory-related effects of fentanyl can be reversed with a non-invasive intervention introduced during early development. These findings can inform ongoing efforts to develop actionable steps toward mitigating the consequences of opioid abuse among pregnant women.

Disclosures: J.B. Alipio: None. **L.M. Riggs:** None. **C. Haga:** None. **M.E. Fox:** None. **K. Arakawa:** None. **R. Balaji:** None. **M. Plank:** None. **N.P. Cramer:** None. **M. Lobo:** None. **A. Keller:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.07

Topic: D.04. Somatosensation – Touch

Support: NINDS/NIH (R01NS094659, R01NS069679)
NSF Graduate Student Research Fellowship

Title: Learning enhances encoding of time and temporal surprise in primary sensory cortex

Authors: *R. RABINOVICH, D. KATO, R. BRUNO;
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Abstract: Primary sensory cortex has long been believed to play a simple role in the initial processing of sensory information. However, cortical activity is influenced by other modalities, task context, reward, and behavioral state; conversely, the superficial layers of cortex tend to respond sparsely to sensory stimulation. We demonstrate that reinforcement learning dramatically alters representations in superficial layers of mouse primary somatosensory cortex. Using in vivo two-photon microscopy of calcium indicators, we longitudinally tracked layer 2/3 neurons as mice learned an object detection task. Learning recruited previously unresponsive neurons, enlarging the neuronal population sensitive to touch (n=10 mice). In contrast, cortical responses habituated upon repeated exposure to unrewarded stimuli (n=7 mice). The ability of a linear decoder (a support vector machine) to identify the stimulus (the presence or absence of the object) and predict the choice (whether or not the mouse displayed anticipatory licking) from population activity improved along with animals' performance. Moreover, training improved population encoding of the passage of time, and unexpected deviations in trial timing elicited even stronger responses than touch did. In conclusion, the superficial layers of sensory cortex exhibit a high degree of learning-dependent plasticity and are strongly modulated by non-sensory but behaviorally-relevant features, such as timing and surprise.

Disclosures: R. Rabinovich: None. **D. Kato:** None. **R. Bruno:** None.

Poster

P413. Barrel Cortex: Integration, Modulation, Learning

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P413.08

Topic: D.04. Somatosensation – Touch

Support: Swiss National Foundation

Title: Rapid re-organisation of layer 2/3 barrel cortex accompanies learning of a whisker detection task

Authors: *A. I. M. IUGA, G. P. FOUSTOUKOS, S. CROCHET, C. C. H. PETERSEN;
Brain Mind Institute, École Polytechnique Fédérale De Lausanne, Lausanne, Switzerland

Abstract: The precise neural circuit mechanisms underlying reward-based learning of a goal-directed sensorimotor transformation remain to be determined. In order to begin to directly investigate this question, we developed a novel single-session learning whisker detection task. This allows for continuous monitoring of behaviour and neuronal activity during the whisker learning process, which may be important for mechanistic insight. In this task, water-restricted

mice are initially trained to lick a reward spout in response to a brief auditory stimulus. Once expert in the auditory detection task, they are presented with interleaved auditory stimuli and single whisker deflections. Over the course of a single recording session, mice learn to associate the novel whisker stimulus with reward availability.Pharmacological and optogenetic inactivation demonstrated the causal contribution of the primary whisker-related somatosensory cortex (wS1) for the acquisition and execution of the task. Consequently, we performed two-photon calcium imaging in order to monitor neuronal activity in wS1 during whisker learning. Our results indicate that a subset of neurons in layer 2/3 of wS1 change their activity across learning. This re-organisation correlates with learning to lick in response to whisker deflection in a reward-dependent manner. Moreover, such re-organisation occurs on a fast time-scale and can be observed during the course of a single training session. High-speed orofacial videography was acquired during the imaging session. Ongoing movement analysis, using DeepLabCut and a generalised linear models, shows that motor movements do not account for the observed changes in neural activity.In summary, we have established a robust single-session reward-based learning paradigm, amenable to detailed investigation of the mechanisms underlying learning and execution of a simple goal-directed sensorimotor transformation.

Disclosures: A.I.M. Iuga: None. **G.P. Foustoukos:** None. **S. Crochet:** None. **C.C.H. Petersen:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.01

Topic: D.05. Olfaction and Taste

Support: NINDS U19NS112953

Title: Neural representations of behavior in olfactory cortex

Authors: *M. SEPPO, A. AOUN, Z. LEVIN, A. PIERRÉ, A. FLEISCHMANN;
Dept. of Neurosci. and the Robert J. and Nancy D. Carney Inst. for Brain Science, Brown Univ., Providence, RI

Abstract: The cerebral cortex transforms sensory information into behavioral outputs. The site and nature of this transformation remains an area of active study. In the olfactory system, odorants activate unique ensembles of neurons in piriform cortex with high variability. We hypothesize that this variability can largely be explained by changes in behavioral state, and that piriform cortex is driven by behavior even under spontaneous conditions, as observed in neocortical brain areas. Here, we investigate this question by performing multiplane, two-photon calcium imaging in the piriform cortex of awake, head-fixed mice during spontaneous and odor-

evoked epochs, while monitoring behavior using videography and physical sensors. Multivariate linear modelling of the activity of individual neurons reveals that activity in piriform cortex is substantially behaviorally driven. Facial movements predict neural activity more than locomotion and sniffing. We find that odor representations occur in ensembles that partially overlap with behavioral ensembles. Future experiments will identify by gene expression and connectivity, neuron types that are preferentially driven by odors or behavior. This work will build towards understanding the functional impact of early sensory-motor integration.

Disclosures: M. Seppo: None. **A. Aoun:** None. **Z. Levin:** None. **A. Pierré:** None. **A. Fleischmann:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.02

Topic: D.05. Olfaction and Taste

Support: HHMI

Title: Statistical learning of odor sequences in primary olfactory cortex

Authors: A. P. FINK[1], R. AXEL[2], ***C. SCHOONOVER**[3];
[1]Neurosci., [2]ZMBBI, [3]Columbia Univ., New York, NY

Abstract: Classical results in experimental psychology (Blodgett/Tolman, Sokolov, Brogden, Groves/Thompson, Berlyne) have documented that animals continuously learn complex features in their sensory environment. This faculty does not depend on external reinforcement to drive learning, only the existence of structure in the world. How does an organism form a model of the world's statistics when the vast majority of the time it is neither rewarded nor punished for doing so? We have developed a behavioral paradigm for the head-fixed mouse that permits observation of exploration and statistical learning simultaneous with longitudinal recording of populations of single units. Mice presented a sequence of two neutral odorants A-B initially investigate the stimuli and then habituate following repeated experience over three days. Recordings in primary olfactory (piriform) cortex show that over this same time period the magnitude of the population response to the A-B sequence decreases markedly, with responses during the B epoch nearly abolished. However, presentation of B alone elicits strong activity; and if B is omitted from the A-B sequence, the piriform responds vigorously to its absence, producing a response that matches the expected timing of the omitted stimulus even though there is no odorant molecule in the nares. Taken together, these concordant behavioral and electrophysiological results show that response magnitude in both behavior and in piriform activity scales inversely with the degree to which an event is predictable. This suggests that activity in piriform does not strictly reflect

physical stimuli, but rather a model of the statistics of the olfactory environment, formed over the course of statistical learning. Familiar stimuli that contain little new information are filtered out, whereas the content of unanticipated events is transmitted to regions downstream.

Disclosures: A.P. Fink: None. **R. Axel:** None. **C. Schoonover:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.03

Topic: D.05. Olfaction and Taste

Support: ONR N000141812002

Title: E:I balance in piriform cortex following olfactory learning

Authors: *K. FRIASON[1], *F. K. E. FRIASON[2], M. B. CANTO-BUSTOS[2,3], A.-M. M. OSWALD[2,4];
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Abstract: The anterior piriform cortex (APC) is thought to be a site of odor encoding. The trilaminar structure and proximity to odor sensory input make the APC highly tractable for dissecting the role of cortical circuitry in sensory processing.
We investigated excitatory-inhibitory (E:I) synaptic plasticity in APC following odor learning. Briefly, mice were trained to discriminate between two overlapping odor mixtures in a digging task. These mice were triple transgenic- expressing a conditional cre-recombinase for targeted recombination in active populations (TRAP); a non-conditional cre-recombinase in either parvalbumin (PV) or somatostatin (SST)-interneurons, and a reporter (tdTOM). These mice also express cre-dependent Channelrhodopsin (ChR2) via viral transfection. Thus, there was conditional expression of ChR2 and tdTom in an ensemble of excitatory neurons that were active during the task, as well as unconditional expression of ChR2 and tdTom in select inhibitory interneurons. Following behavioral training, we targeted these APC population neurons for recording and/or reactivation in vitro.
We performed voltage clamp recordings from ensemble (tdTom+) and non-ensemble (tdTom-) PNs while electrically stimulating the intracortical tract that recruits recurrent excitation and inhibition. PNs were held at -50 mV or >0 mV to isolate excitatory post-synaptic currents (EPSCs) and inhibitory pre-synaptic currents (IPSCs) respectively. After training, EPSCs and IPSCs both showed significantly increased amplitudes in tdTom(+) ensemble PNs compared to PNs outside the ensemble (tdTom-). The increase in excitation is balanced by the increase of

inhibition as the two positively scale together. Since inhibition is mediated by a diverse cell population, we further investigated how inhibition mediated by two classes of inhibitory neuron, PV and SST, scales with excitation. To answer this, we recorded EPSC and IPSC responses in tdTom(+/-) PNs in response to light activation of ChR2+ PNs and PV or SST interneurons. Preliminary findings suggest that inhibition mediated by both PV and SST interneurons scales with excitation. While increases in PV-mediated inhibition appear to be selective for active ensemble neurons (tdTom), changes in SST-mediated inhibition are more broadly distributed. Taken together, our findings suggest that both classes of interneuron may play a role in balancing changes in excitation following odor task learning.

Disclosures: F.K.E. Friason: None. **M.B. Canto-Bustos:** None. **A.M. Oswald:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.04

Topic: D.05. Olfaction and Taste

Support: NINDS U19NS112953
5R01DC017437-03

Title: Information processing by distinct projection neuron types in the mouse olfactory cortex

Authors: *S. DASTE, A. FLEISCHMANN;
Dept. of Neurosci. and the Robert J. and Nancy D. Carney Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: Sensory cortexes contain a multitude of neuron types projecting to different target areas. Determining their distinct functional properties is essential to better understand how stimulus information is transmitted across brain areas. Piriform cortex (PCx), the main recipient of sensory input from the olfactory bulb, contains neurons that project to numerous areas, including the Olfactory Bulb (PCx_{OB}) and the medial Prefrontal Cortex (PCx_{mPFC}). Both PCx_{OB} and PCx_{mPFC} cells are deep-layer pyramidal cells, however, whether they selectively encode distinct stimulus information is unknown. Here, we record neuronal activity in the PCx of awake behaving male and female mice (4-6 months old) using GRIN lens technology and two-photon calcium imaging microscopy. We label PCx_{OB} and PCx_{mPFC} subpopulations using retrogradely transported Adeno-associated virus, and we determine their response properties under passive odor presentation. We show that PCx_{OB} and PCx_{mPFC} subpopulations (n=5 mice each) can accurately be identified based on their odor response profiles. Moreover, odor identity is more efficiently encoded in PCx_{mPFC} neurons compared to PCx_{OB} neurons. Our results suggest that

odor information coding differs between PCx neuron types, providing new insights into how olfactory information is routed across brain areas.

Disclosures: S. Daste: None. **A. Fleischmann:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.05

Topic: D.05. Olfaction and Taste

Support: R01 Grant DC018032
Alfred P. Sloan Foundation
Rackham Graduate Student Research Grant

Title: Re-engineering development to test models of sensory coding

Authors: *M. AHMED, E. A. PEDERSEN, D. L. WILLIAMS, E. J. CLOWNEY;
Dept. of Molecular, Cell. and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: We experience countless sensory cues on a day-to-day basis. How is the brain wired to deal with this wide and unpredictable array of stimuli with only a limited set of neurons? To do this, principal neurons of sensory perception and associative learning regions receive information about cues in combinations. There is an expansion of inputs from a small set of neurons onto a much larger set of postsynaptic cells, and each postsynaptic cell only receives a few inputs (average of ~5). Such sparse, combinatorial innervation is seen in multiple perceptual and learning centers such as the cerebellum, the hippocampus, and the vertebrate and fly olfactory systems. Theoretically, the density and complexity of sensory inputs to principal neurons of these regions optimize the tradeoff between stimulus representation and discrimination. However, this has not been tested at the functional level. To test this relationship experimentally, we are using the *Drosophila* mushroom body (MB) as our model system, as it is the simplest and best studied of these associative learning regions. Within the MB, individual Kenyon cells (KCs) receive combinatorial inputs from a few olfactory projections neurons (PNs) to represent odors. To determine the relation between circuit form and function *in vivo*, we have developed a suite of chemical and genetic methods to manipulate MB cell repertoires during development. In our work to date, we find that: (1) sparseness of PN inputs to KCs is robust to changes in the size of PN and KC populations, (2) KCs orchestrate connectivity density: the number of dendrites they produce during development is relatively inflexible, while olfactory PNs adjust their production of presynaptic sites several fold to adjust to developmental perturbations, and (3) when sparseness is preserved, KC ability to represent odors is largely intact. Seeing that the KC dendrite number is largely unaltered across all the manipulations

supports the significance of the number of inputs received by KCs. Going forward, we are developing ways to “break” the sparse innervation of KCs by PNs by directly changing the number of dendrites produced by KCs. This will allow us to test *in vivo* the role for sparse innervation in sensory coding and associative learning. Ultimately, this work will not only contribute to our understanding of the connectivity architecture underlying sensory coding, but also allow us to manipulate development to shape object representation in the brain.

Disclosures: M. Ahmed: None. **E.A. Pedersen:** None. **D.L. Williams:** None. **E.J. Clowney:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.06

Topic: D.05. Olfaction and Taste

Support: NIA AG-049937A

Title: Gabaergic innervation of the olfactory cortex by basal forebrain long-range projecting neurons

Authors: *R. C. ARANEDA[1], P. S. VILLAR[2];
[1]Univ. of Maryalnd, College Park, MD; [2]Biol., Univ. of Maryland, College Park, MD

Abstract: Sensory perception requires the coordination of sizable neuronal assemblies across multiple brain regions; yet, how such cooperation is achieved is not understood. Previous studies have shown that long-range projecting neurons influence the activity of interconnected regions by modulating specific circuit domains and are necessary for sensory binding and memory formation. Particularly, the processing of early sensory signals in the olfactory bulb (OB) is highly regulated by basal forebrain long-range GABAergic neurons (BF-LRGN). This GABAergic afference influences the OB output by acting on local GABAergic circuits. Activation of BF-LRGNs produces an overall desinhibitory effect on the output neurons, decreasing their firing precision, and reducing the intensity of local oscillatory activity. Furthermore, BF-LRGNs also innervate higher order targets in the olfactory pathway, including the olfactory cortex, however the physiological properties and function of these GABAergic projections remain unknown. Here, using conditional genetics and confocal microscopy we studied the distribution of BF-LRGN projections to the piriform cortex (PC). We found that the BF-LRGN innervation was highest in layer 1 of PC (norm. pixel intensity 0.72 ± 0.01, n= 7 slices, 2 mice) and layer 3 (norm. pixel intensity 0.46 ± 0.001, n= 7 slices, 2 mice), where abundant axonal boutons can be observed. In contrast, layer 2 of PC, which contains the highest number of pyramidal neurons, exhibited a lower degree of innervation (norm. pixel intensity 0.35

± 0.001, n= 7 slices, 2 mice), suggesting a differential influence of BF inhibition across the PC cellular layers. Moreover, using whole cell electrophysiology in brain slices and optogenetics we examined the postsynaptic targets of BF-LRGNs in the PC. Surprisingly, we found that light stimulation of LRGN expressing channelrhodopsin-2 consistently evoked short latency (~5 ms) responses in all cortical layers of the PC (layer 1: -3.2 ± 2.2 pC, n= 2; layer 2: -20 ± 5 pC, n= 3; layer 3: -4.5 ± 2 pC, n= 2). These results suggest that BF GABAergic projections are well poised to influence the activity of local GABAergic cells as well pyramidal cell function in the PC through fast inhibitory transmission.

Disclosures: R.C. Araneda: None. **P.S. Villar:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.07

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC014701
NIH Grant DC014367

Title: A general theory of olfactory learning and representation

Authors: *T. A. CLELAND[1], J. A. COOK[2], A. BORTHAKUR[3];
[1]Psychology, [2]Mathematics, [3]Computat. Biol., Cornell Univ., Ithaca, NY

Abstract: Theories of sensory representation must consider not only physiological response properties that are sensitive to changing stimuli, but also a broader set of factors such as robustness to natural interference and how the properties of individual circuit elements can be coordinated into a functional system. Broadly, we propose that odor representations are embedded in spike timing-dependent representations on the gamma/beta timescale, which govern dynamic neuronal interactions and plasticity within the deep olfactory bulb (OB) and between the OB and its follower cortices. We first describe how this architecture can be mapped to a generalized geometric framework for odor category learning, using the tools of smooth manifold theory. Beginning with the space of all possible physical inputs to the olfactory system (the irreducibly high-dimensional R-space), we incorporate a dynamic model for perceptual learning to transform this input space into a smooth, pliable perceptual space termed S-space. S-space embeds R-space, but also admits the capacity for experience-dependent category learning, progressively developing hierarchical, quasi-discrete categories (corresponding to learned odors) atop an intact physical similarity metric. This framework yields interpretable depictions of behavioral phenomena such as stimulus generalization and the speed-accuracy trade-off. Importantly, this odor learning depends on locally-governed synaptic plasticity within olfactory

bulb, indicating that geometries that fix curvature (whether spherical, hyperbolic, or otherwise) must be ruled out in favor of a flexible geometric framework that can be described using the theory of sheaves. In computational models based upon these principles, spike timing-dependent plasticity at the mitral-granule cell synapse generates highly configural feature detectors that iteratively modify the spike timing-dependent activity of olfactory bulb principal neurons. The resulting attractor dynamics are able to denoise raw chemosensory inputs in which known odors of interest are substantially occluded by background odorants that compete for the same receptors, thereby disrupting the cross-receptor pattern on which odor recognition ostensibly depends. This model also exhibits rapid online learning (in which subsequent learning does not disrupt existing representations), and suggests a necessary role for adult neurogenesis as an efficient mechanism to enable lifelong learning of meaningful odors.

Disclosures: T.A. Cleland: None. **J.A. Cook:** None. **A. Borthakur:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.08

Topic: D.05. Olfaction and Taste

Support: NIH R01 MH113924
NSF CAREER 1749772
Cystinosis Research Foundation
Kilian J. and Caroline F. Schmitt Foundation

Title: Top-down feedback gates flexible coding strategies in olfactory cortex

Authors: *Z. CHEN[1], K. PADMANABHAN[2];
[1]Brain and Cognitive Sci., [2]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: In chemical sensation, multiple models have been proposed as to how odors are represented by patterns of neuronal activity in the olfactory cortex. One hypothesis is that the combinatorial patterns of activity restricted within specific sniff-related time windows are critical for encoding information about odors, while another proposes that the patterns of neural activity evolve across time, and it is this temporal structure that is essential for encoding odor information. Each model has experimental support and draws upon theoretical frameworks that make them appealing, but it remains unclear how these different models encode odor information, and the extent to which these are different instantiations of a single overarching strategy of computing. We hypothesize that different findings on how piriform cortex codes for odor information can be reconciled by examining the role of feedback from piriform cortex to the bulb. To test this hypothesis, we built a realistic spiking neuronal network model which

recapitulated the circuit architecture within and between the main olfactory bulb and piriform cortex and studied how centrifugal feedback influenced odor information encoded by the piriform cell population. We found that top-down feedback to the olfactory bulb shaped the information transmitted to the olfactory cortex by switching between these two models. Using a detailed model of the early olfactory system, we demonstrated that feedback control of inhibitory granule cell activity in the main olfactory bulb restructured the firing patterns of piriform cortical cells across time. This translated to performance gains in both the accuracy and reaction time of odor discrimination tasks. Taken together we propose a new framework for early olfactory computation, one in which top-down feedback to the bulb flexibly gates the temporal structure of neural activity in olfactory cortex, allowing the early olfactory system to dynamically switch between combinatorial codes and temporal codes.

Disclosures: Z. Chen: None. **K. Padmanabhan:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.09

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01DC016289
NIH Grant R01DC017311

Title: Neural representation of odor mixtures in the piriform cortex evolves to balance identity coding and learned categorization

Authors: *A. BERNERS-LEE[1], E. SHTRAHMAN[1], J. GRIMAUD[2], V. N. MURTHY[1]; [1]Dept. of Mol. and Cell. Biol. and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]École de biotechnologies, Sup'Biotech, Villejuif, France

Abstract: Animals and the neural circuits that support their behavior have a natural trade-off between categorized and unique representations of stimuli. We investigated how learned odor mixtures are encoded and evolve in the posterior piriform cortex of mice while they learn to discriminate a unique target odor mixture against hundreds of nontarget mixtures. We find that a significant proportion of piriform neurons discriminate between target and nontarget odor mixtures, and that neurons that prefer the target odor tend to respond with brief increases in firing rate at odor onset compared to sustained and/or decreased firing. Mice also learn that new probe odors, which contain a mixture of target and nontarget components, are nontarget odors. Although a portion of neurons distinguish between probe and non-target odor mixtures and between different non-target mixtures, there are significantly fewer of these neurons than there are target-discriminating neurons. In addition, we analyze the population vector (PV)

correlations across trial types and find that the population representation for these mixtures change differently across sessions. Probe PVs tend to become more similar to nontarget PVs over sessions, while individual repeated nontarget mixtures tend to become more similar to themselves (the representation becoming more distinct or unique). Although probe and repeated nontarget trial types change their PV representations in different ways, they both allow for better population decoding of the category decision (target/non-target). These data show that populations of piriform neurons are able to change their representation of complex stimuli in different ways to balance categorical and identity encoding in a manner that may be useful for learning in a changing environment.

Disclosures: A. Berners-Lee: None. **E. Shtrahman:** None. **J. Grimaud:** None. **V.N. Murthy:** None.

Poster

P422. Olfactory Coding: Higher Order Processing I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P422.10

Topic: D.05. Olfaction and Taste

Support: MH084989

Title: Requirements for experience-dependent PKA activity in brain learning and memory circuitry

Authors: *J. C. SEARS[1], K. S. BROADIE[2];
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Abstract: Protein Kinase A (PKA) activity drives learning and memory plasticity, but PKA circuit requirements and disruption in neurological disorders remain to be determined. Our goal is to test PKA mechanism in the *Drosophila* central brain Mushroom Body (MB) learning and memory circuitry, which projects via distinct lobes that have subdivided innervation patterns mapping to behaviorally-relevant output neurons. Our previous work demonstrates that both Fragile X Mental Retardation Protein (FMRP) and autism-associated PKA-anchor Rugose (Neurobeachin homolog; NBEA) promote PKA activity in MB Kenyon cells. Using a PKA activity biosensor that forms reversible GFP foci upon PKA phosphorylation (PKA-SPARK), we find young animals (0 days post-eclosion: 0 dpe) display almost no lobe PKA-SPARK foci, whereas 7 dpe animals display striking PKA-SPARK activity localized to two distinct circuit nodes. This increased signaling occurs more strongly in females relative to males, and loss of phosphodiesterase Dunce/PDE4 results in reduced PKA-SPARK activity. This acute onset of PKA activity suggests early experience-dependent signaling in specific MB circuits connections. Consistent with this hypothesis, rearing in reduced sensory stimuli environments results in much

less PKA activity. Moreover, removal of FMRP and Rugose/NBEA results in drastically reduced PKA activity, also suggesting disruption of experience-driven signaling. Taken together, these results define a new paradigm for testing the experience-dependent requirements for PKA activity in brain learning and memory circuitry. Ongoing work focuses on MB circuit mapping of this PKA signaling mechanism, testing early-life critical period requirements, and combining Fragile X syndrome and other ASD disease models with variable sensory environment manipulations.

Disclosures: J.C. Sears: None. **K.S. Broadie:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.01

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit 01 CX001812
NYS SCIRB C32236GG
NYS SCIRB C33279GG
Stratton VA Medical Center

Title: Identifying and redressing H-reflex contamination by the M response in the flexor carpi radialis muscle

Authors: *J. S. CARP[1,2], M. A. LASKY[1,4], R. A. MARINELLI[1,4], J. H. BARNES[1,5], J. A. BRANGACCIO[1], J. R. WOLPAW[1,2,3], J. J. S. NORTON[1,3];
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Abstract: The H-reflex (HR) is an important tool for experimental evaluation of spinal excitability, neurological diagnosis, and new rehabilitation strategies. Assessment of HR size is straightforward in distal muscles, e.g., soleus (SOL), where there is little or no temporal overlap of the direct muscle response (MR) and the HR, but can be problematic in more proximal muscles, e.g., flexor carpi radialis (FCR), where there can be substantial overlap between MR and HR.
To assess HR contamination by the MR (study approved by Stratton VA IRB), we recorded electromyographic responses evoked over a wide range of stimulation currents in the SOL (n=15) and FCR (n=11) muscles of 26 neurologically normal subjects. MR and HR sizes were calculated as mean rectified amplitudes within time windows starting at the onset of the earliest

large phase of each response and ending at the offset of the adjacent large phase of the opposite polarity. Visual inspection of the responses evoked over a wide stimulus range revealed contamination of the HR by the MR in all FCR, but not SOL. Recruitment curves constructed from the individual trials showed the failure of the HR to be fully suppressed at the stimulus current that elicited the maximum MR (Mmax) in FCR, but not in SOL (mean H at Mmax±SD (as % of Mmax)=21±8% and 8±4%, respectively). After limiting the HR window to the second (i.e., longer-latency) of the largest phases of the HR, the HR at Mmax of FCR and SOL were comparable to each other (mean±SD=10±6% and 6±5%, respectively) and to that of SOL using the full analysis window. To validate that limiting the HR window to the second large phase of the HR reduces contamination by the MR, we evaluated the correlation between the two largest oppositely signed phases of the HR. We hypothesized that contamination of the HR by the MR in the FCR would be manifested as a deviation from symmetric growth with increasing current. In fact, the largest positive and negative phases of the HR were highly correlated in SOL (mean r±SD=0.94±0.10; min/max=0.66/1.00), but were poorly correlated in FCR (mean r±SD=0.42±0.33; min/max=-0.09/0.87) (SOL≠FCR by t-test, $p<0.01$). In FCR, the phases were highly correlated up to 1.04±0.04 times the current that elicited the maximum HR, but this linear relationship deteriorated as current was further increased.
These results suggest that the FCR (but not SOL) HR is frequently contaminated by the MR at high stimulation levels. We propose that restricting the time window in which the HR size is evaluated to the second large phase of the HR can reduce this contamination. This should facilitate generation of more accurate data, which can benefit experimental and translational studies and clinical practice.

Disclosures: J.S. Carp: None. **M.A. Lasky:** None. **R.A. Marinelli:** None. **J.H. Barnes:** None. **J.A. Brangaccio:** None. **J.R. Wolpaw:** None. **J.J.S. Norton:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.02

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit I01 CX001812
NYS SCIRB C32236GG and C33279GG
Stratton VA Medical Center

Title: Flexor carpi radialis H-reflex operant conditioning

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Abstract: H-reflex operant conditioning is a promising new method for improving motor control impaired by incomplete spinal cord injury (iSCI) or other neurological injury or disease (Thompson et al., J Neurosci, 2013). In this protocol, participants learn to increase (up-condition) or decrease (down-condition) H-reflex size. In people with spasticity due to iSCI, H-reflex down-conditioning can trigger widespread beneficial plasticity that improves movement. H-reflex operant conditioning is non-invasive, and has no known adverse side effects; it can complement existing therapies and enhance functional recovery (Norton and Wolpaw, COBS, 2018). Previous studies have operantly conditioned the H-reflex of the soleus muscle in the leg (Thompson et al., J Neurosci, 2009; Thompson et al., J Neurosci, 2013). We are now extending H-reflex operant conditioning to the arm, specifically to the forearm muscle flexor carpi radialis (FCR).
The protocol is based on the leg protocol described by Thompson et al. (J Neurosci; 2009). In this first arm study, participants without neurological impairment complete 6 baseline (i.e., control) and 24 conditioning sessions (1 hr/session; 3/wk X 10 wks). Participants maintain a predefined level of FCR EMG activity prior to H-reflex elicitation by a stimulus that is continually adjusted to maintain a predefined M-wave size. Concurrent EEG data are recorded every sixth session. In control trials, the participant receives no feedback on H-reflex size; in conditioning trials, s/he receives immediate reward if H-reflex size is below (down-conditioning) or above (up-conditioning) a criterion.
To date, 13 healthy participants (6 down- and 7 up-conditioned) have completed the protocol; and we have analyzed the data from 11 of these participants. A comparison of the ratio of the H-reflex size from the 6 baseline sessions to the average H-reflex size from the last 6 conditioning sessions shows that eight of the 11 participants successfully changed their reflex size in the instructed direction (t-test). In one of the other three participants, H-reflex size did not change; in two participants, the H-reflex size changed, but not in the desired direction. Data indicate that operant conditioning of the FCR H-reflex is possible. Following confirmation of these initial results and further development, we plan to investigate FCR H-reflex conditioning for motor recovery in Veterans who have had a stroke.

Disclosures: J.J.S. Norton: None. **S. Devetzoglou-Toliou:** None. **J.A. Brangaccio:** None. **D.E. Gemoets:** None. **D. Hahn:** None. **S.M. Heckman:** None. **T.M. Vaughan:** None. **J.S. Carp:** None. **J.R. Wolpaw:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.03

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit I01 CX001812
NYS SCIRB C32236GG
C33279GG Stratton VA Medical Center

Title: A BCI-enhanced H-reflex Operant Conditioning System

Authors: *S. DEVETZOGLOU TOLIOU[1,3], J. P. MCLINDEN[5], M. ADAMEK[1,6], J. A. BRANGACCIO[1], J. S. CARP[1], J. HILL[1], J. J. S. NORTON[1,3], Y. SHAHRIARI[5], J. R. WOLPAW[2,4];
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Abstract: H-reflex operant conditioning (OC) can improve motor function following spinal cord injury (SCI) and potentially stroke and other neuromuscular disorders (*Thompson et al., J Neurosci, 2013*). Thompson et al. (2018) showed that, following brain-computer interface (BCI) training to control the amplitude of EEG mu (8-13Hz) or beta (15-30Hz) sensorimotor rhythm (SMR) activity, H-reflex size correlated with this amplitude.
Encouraged by this work, we are developing a BCI-enhanced H-reflex OC system. This system simultaneously records EMG and EEG. In standard H-reflex OC, the H-reflex is elicited when the participant maintains a predefined level of EMG activity for at least two sec. In the BCI-enhanced system, the H-reflex is elicited when the participant satisfies pre-defined criteria for both EMG and SMR levels.
Our BCI-enhanced H-reflex OC system combines BCI2000 (*Schalk et al. IEEE TBME, 2004*) and the Evoked Potential Operant Conditioning System (EPOCS). BCI2000 records EEG data and extracts SMR features (i.e., mu and beta amplitudes). The SMR features are transmitted to EPOCS through the BCI2000 AppConnector. EPOCS records and processes the EMG activity and provides feedback to the participant on both EMG and SMR levels.
As an initial test of the functionality of the combined system, we studied two participants. The Stratton VAMC IRB approved the study. We first trained (~8 sessions over 6 weeks) each participant to modulate SMR amplitude to select targets on a computer screen (*Wolpaw et al., Clin Neurophysiol, 1991*) with >70% accuracy (chance: 50%). We also completed two H-reflex screening sessions to identify optimal sites to stimulate and record the H-reflex of the flexor carpi radialis muscle. The participant then completed two H-reflex OC sessions. Each session comprised 100 standard H-reflex conditioning trials and 100 BCI-enhanced H-reflex conditioning trials (i.e., SMR above a criterion value).
The initial results: (1) show that the combined BCI2000/EPOCs OC system works smoothly online; and (2) suggest that SMR activity in the 1 sec before H-reflex elicitation is higher with the BCI-enhanced system. If additional data confirm these results, we will begin to assess whether the BCI-enhanced OC system can increase the rate, magnitude, and/or reliability of H-reflex operant conditioning, and can thereby increase the ability of this new rehabilitation

method to complement other therapies and augment recovery of useful function after stroke, spinal cord injury, or other neuromuscular disorders.

Disclosures: S. Devetzoglou Toliou: None. **J.P. McLinden:** None. **M. Adamek:** None. **J.A. Brangaccio:** None. **J.S. Carp:** None. **J. Hill:** None. **J.J.S. Norton:** None. **Y. Shahriari:** None. **J.R. Wolpaw:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.04

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit 01 CX001812
NYS SCIRB C32236GG and C33279GG
Stratton VA Medical Center

Title: Separating overlapping M-wave and H-reflex components of spinal evoked potentials

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Abstract: The Hoffmann reflex (HR) is the electrically induced analog of the spinal stretch reflex. It is an important tool to assess the modulation of spinal reflex activity, and it is useful for motor rehabilitation research in people with spinal cord injury. At low-intensity stimulation, efferent action potentials travel from the alpha motor neuron to the muscle (recorded as HR), and at higher intensities, they also travel directly to the muscle (recorded as M-wave (M)) and antidromically to the motor neuron, colliding with the HR. At supramaximal intensity, as M reaches M_{max}, the HR is largely cancelled. In distal muscles, such as the soleus (SO), this is typically reflected in recruitment curve measurements where the HR gradually increases with increasing stimulation intensity, and, then, decreases to a small percentage of M_{max} at supramaximal intensity. In more proximal muscles, such as the flexor carpi radialis (FCR), the M-wave substantially overlaps the HR at higher intensities, increasing the H measurements and distorting the curve. We hypothesize that mathematically separating these waves with a blind source separation algorithm-constrained independent component analysis (cICA), could eliminate M contamination of the HR at higher stimulation levels.
We use single-channel EMG from FCR (n=9) and SO (n=15), eliciting HR and M from stimulation of the median and the posterior tibial nerve, respectively. Data was collected with

informed consent; Stratton IRB approved this study. Responses were captured over a wide range of stimulation intensities, 4 trials/intensity, 5-s interstimulus interval. Epochs (0-60ms) were created relative to stimulation onset. Area under the curve was calculated for predefined M and H response windows, per stimulation intensity. These time-windows were marked manually, based on trials where these responses were least contaminated; the same trials were also used to construct the reference signals for the cICA. Two statistically independent components that matched the reference signal morphologies. They were projected back to the EMG signal space to obtain the de-contaminated trials. Contamination at high intensities is evident in raw FCR data, where H at M_{max} (as % of M_{max}) was 28.7$\pm$6.9 (mean+SD), compared to 8.3$\pm$4.1 for the less contaminated SO. cICA reduced it significantly for FCR to 9.6$\pm$7.6, $p < 0.01$ (Mann-Whitney U test), bringing it to a more typical range like the soleus. As expected, it did not reduce soleus H at M_{max} significantly (5.4$\pm$4, $p > 0.01$). M_{max}, and H and M responses at lower intensities remained largely unchanged. This preliminary analysis demonstrates the cICA based improvement in HR evaluation for proximal muscles.

Disclosures: D. Gupta: None. **J.S. Carp:** None. **J. Barnes:** None. **J.S. Norton:** None. **J. Hill:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.05

Topic: E.06. Posture and Gait

Support: NIH NINDS U44NS114420
NIH NIBIB P41 EB018783
VA Merit 01 CX001812
NYS SCIRB C33279GG
Stratton VA Medical Center

Title: Noninvasive, multielectrode array-based platform for operant conditioning of soleus H-reflex

Authors: *M. MCKINNON[1], N. HILL[2,3], A. PREYER[1], M. GOEBEL[1], D. HOCHMAN[1], J. NORTON[2,3], J. CARP[2,4], J. WOLPAW[2,3,4], A. K. THOMPSON[5], I. CLEMENTS[1]; [1]BioCircuit Technologies, Atlanta, GA; [2]Natl. Ctr. for Adaptive Neurotechnologies, Albany Stratton VA Med. Ctr., Albany, NY; [3]Dept. of Electrical and Computer Engin., [4]Sch. of Publ. Hlth., Univ. at Albany, State Univ. of New York, Albany, NY; [5]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: Spinal cord injury can produce spasticity and impair locomotion; operant conditioning of the soleus H-reflex can reduce spasticity and improve locomotion. At present, this conditioning is highly specialized and technically challenging, hindering widespread dissemination of this powerful therapy. We are developing a clinically practical system for semi-automated H-reflex operant conditioning by leveraging the capabilities of transcutaneous multielectrode arrays for both stimulation and recording. At present, inter- and intra-subject variability makes automation of this therapy difficult. Here we explore how array-based processing techniques can reduce sources of extrinsic variability and thereby improve the robustness of automation.
Flexible, adhesive arrays of hydrogel electrodes enabled both targeted stimulation of the tibial nerve and high-density surface EMG recording of the resulting M- and H-waves at the soleus. We obtained soleus H-reflex/M-wave recruitment data from healthy volunteers of either sex. EMG signals were amplified and digitized with a custom 32-channel recording device that provides high quality, low-noise electrophysiological data. Custom software was used to visualize the processed data.
Multielectrode recording arrays cover a larger area of the soleus with higher spatial resolution compared to standard bipolar electrodes, providing a more comprehensive picture of soleus recruitment. A novel optimization algorithm uses this information to automatically correct electrode misplacement for a given person on subsequent days. A wavelet-based approach to automatically detect the H-reflex and reliably determine its amplitude was developed and tested on manually curated clinical datasets. This approach was validated on novel datasets and showed similar performance to expertly curated data. A separate multielectrode array designed for stimulation allows selective targeting of the tibial nerve while minimizing discomfort. Algorithms for automatically selecting the optimal stimulation parameters are in development. These experimental results suggest that this combination of multichannel stimulation and multielectrode recording in a single platform will serve clinicians who wish to perform operant conditioning therapy. Furthermore, this platform may be useful for clinicians and clinical researchers interested in targeted muscle stimulation and high-resolution EMG recording for additional applications beyond the soleus H-reflex conditioning.

Disclosures: **M. McKinnon:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **N. Hill:** None. **A. Preyer:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **M. Goebel:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **D. Hochman:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **J. Norton:** None. **J. Carp:** None. **J. Wolpaw:** None. **A.K. Thompson:** None. **I. Clements:** A. Employment/Salary (full or part-time):; BioCircuit Technologies.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.06

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844-01 (Kautz);
NIBIB 1P41EB018783 (Wolpaw)
NINDS NS114279 (Thompson)
NYS SCIRB C33279GG
The Doscher Neurorehabilitation Research Program (Thompson)
South Carolina Spinal Cord Injury Research Fund (SCIRF#2014 R01, #2019 PD-01, Thompson)
Stratton VA Medical Center

Title: The excitability of soleus H-reflex pathway during standing in the paretic and non-paretic legs of people with hemiparesis due to stroke: initial studies.

Authors: *J. A. BRANGACCIO[1], A. M. PHIPPS[2], J. R. WOLPAW[1,3], A. K. THOMPSON[2]; [1]Natl. Ctr. for Adaptive Neurotechnologies, Stratton VA Med. Ctr., Albany, NY; [2]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC; [3]Sch. Pub. Hlth., St Univ. NY, Albany, NY

Abstract: Spasticity and spastic hyperreflexia are common in people after stroke. The role of spasticity in post-stroke gait disorders is uncertain. Neurophysiological studies often exam only the paretic side. However, unilateral measures alone are probably not sufficient for evaluating post-stroke movement disorders and impaired motor control. Thus, in this study, we measured the soleus H-reflex during standing in paretic and non-paretic legs of individuals with hemiparesis due to stroke, and in both legs of individuals with no known neurological conditions. The MUSC IRB approved this study. In 11 individuals with hemiparesis due to stroke and 7 individuals without stroke, soleus H-reflex/M-wave recruitment curves were measured bilaterally during standing. Soleus and TA EMG levels were recorded bilaterally and a stable soleus background EMG level was maintained throughout measurement (typically 5-18 μV) in the test leg. Tibial nerve stimulus intensity increased from just below H-reflex threshold to an intensity just above that needed to elicit the maximum M-wave (Mmax). Two-to-four responses were obtained at each intensity. In people after stroke, peak-to-peak maximum H-reflex (Hmax) in the paretic leg was (3.39± 1.78mV, 53% $\pm$ 19 %Mmax), significantly larger (p=.03 by paired t-test) than that in non-paretic leg (2.11± .83mV, 33% ± 15%Mmax). During the paretic leg H-reflex measurement, the contralateral (non-paretic) TA was more active than the paretic TA (p=.01 by paired t-test); whereas during the non-paretic H-reflex measurement, TA was similarly active in both the non-paretic and paretic legs (p=.63 by paired t-test). Among individuals without stroke, the side-to-side difference in H-reflex size averaged (10% ±11%Mmax); and TA activity was minimal in both legs. The results indicate that the standing H-reflex after stroke is larger on the paretic side than the non-paretic side. While maintaining moderate soleus activity during standing, those with stroke showed increased TA activity in both the paretic and non-paretic leg. These results suggest: 1) stroke is not a unilateral event, EMG during standing is altered in both legs; 2) a paretic TA (producing foot drop) may be activated in standing. Further studies are underway to better understand the neurophysiological mechanisms and functional impact of side-to-side differences in spinal reflexes post-stroke.

Disclosures: J.A. Brangaccio: None. **A.M. Phipps:** None. **J.R. Wolpaw:** None. **A.K. Thompson:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.07

Topic: E.06. Posture and Gait

Support: NSF DGE 0718128
ONR ETOWL program
NIH AG030815
Dr. Benno Nigg Chair, NSERC (Discovery and CRC Tier I)
NSERC Discovery Grant

Title: The Effect of Postural Task on Lower-Limb Reflex Excitability

Authors: *O. DARICI, H. HODGSON, S. AJAO, T. BUTT, A. D. KUO, R. M. PETERS; Fac. of Kinesiology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Reflex excitability is traditionally assessed as the muscle response to transient electrical nerve stimuli (Hoffman, or H-reflex). An alternative is Noisy Tendon Vibration (NTV), where the tendon is vibrated with a noisy signal to induce electromyography (EMG) responses, quantified by its correlation with stimulus. Excitability can thus be assessed continuously throughout a movement without inducing discrete perturbations. We sought to test whether NTV yields different postural excitability than H-reflex, which does not mechanically activate muscle spindle afferents that may be critical to actual reflexes. We measured NTV in three different postural conditions: quiet standing, standing while holding a support bar (to reduce lower limb proprioceptive input), and sitting. Previous H-reflex results show that holding a support bar diminishes responses compared to quiet standing, whereas sitting enhances responses. We applied NTV with a custom-developed vibrator, using white noise stimulus (bandwidth 0-100 Hz) to the right Achilles tendon of 5 healthy adult subjects, recording vibration motor acceleration as the input stimulus. Output was recorded as EMG from soleus, medial gastrocnemius, and tibialis anterior. Frequency- and time-domain correlation analysis (coherence and cumulant density, respectively) was performed between EMG and acceleration data (3 min per trial). Center of pressure (COP) trajectory was used to quantify posture variability. We found significant input-output correlation at 40-100 Hz for all conditions (coherence, p;<0.05), indicating the main frequency range of reflexes. Correlation was 90% smaller for sitting compared to standing (coherence, p;<0.05), and 52% smaller with support bar compared to standing (p;<0.05). Time domain (cumulant density plots) responses exhibited peaks at around 40 ms, indicating relatively short latency reflexes, regardless of the task. The

support bar condition yielded decreased anterior-posterior COP compared to standing (p;<0.05), suggesting less reliance on the ankle for balance, and thus lower coherence. NTV indicates reduced reflex excitability for sitting and holding the support bar, compared to standing. This could be due to presynaptic reflex inhibition and/or other competing inputs to the motor neuron pool. The ankle may rely less on spindle afferents when not actively maintaining balance. NTV can quantify reflexes continuously and may yield information complementary to H reflexes, with respective advantages yet to be tested thoroughly.

Disclosures: O. Darici: None. **H. Hodgson:** None. **S. Ajao:** None. **T. Butt:** None. **A.D. Kuo:** None. **R.M. Peters:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.08

Topic: E.06. Posture and Gait

Title: The rat tail musculature as a hyper-redundant model for evaluating the role of muscle synergies, especially the co-contraction synergy

Authors: *C. L. CLELAND[1], A. C. KAHLE[2], L. HARRIS[2], E. DICKSON[2], A. PEROE[2], T. TOLLEY[2], L. JAGANNATHAN[2], A. ADAWI[2];
[2]Biol., [1]James Madison Univ., Harrisonburg, VA

Abstract: A central question in motor control is how the CNS overcomes the computational challenges of movement planning. Synergies have been proposed as one solution, which are effectively studied in hyper-redundant systems such as the octopus arm. We propose the rat tail as an ideal mammalian system to explore synergies, since the combination of external muscles and muscle fascicles (in the pelvis with long tendons extending into the tail), and the intrinsic muscles (within the tail), approach 300 in number, creating extensive muscular redundancy. The specific aim of our research is to use four complementary approaches - tail EMG, tendon lesions, movement reconstruction, and computer biomechanical simulation - to determine the spatial-temporal pattern of tail muscle activity during the NWR, with the broader goal of identifying and characterizing muscles synergies, especially the co-contraction synergy. For the four approaches; EMG: eight extrinsic and/or intrinsic rat tail muscles were selectively recorded during the heat-evoked tail NWR. LESION: Extrinsic tendons were sectioned so that the NWR could be compared before and after the lesions. RECONSTRUCTION: The behavioral NWR was first characterized by high speed video. Next, under anesthesia, individual tail muscle tendons were manually pulled and the tail movement video recorded. Finally, tail movement primitives were computationally combined to identify the potential pattern of muscle activity underlying the NWR movement. SIMULATION: A biomechanical model of the tail was developed in Simulink

to identify the torques sufficient to create movement that matched heat-evoked tail NWR behavior. In the extrinsic muscles, agonist activity as expected, preceded antagonist activity, but only slightly (10 ms), with the agonist and antagonist muscles co-activated over most of the movement. Non-negative matrix factorization suggested that nearly half of the EMG could be accounted for by a co-contraction synergy. In further support, lesions of all lateral extrinsic tendons resulted in movement in the opposite direction, into the stimulus. In contrast, the intrinsic muscles showed nearly simultaneous activity, consistent with predominant co-activation. Reconstruction and simulation studies suggest that small tail movements are accomplished by only a few tendons acting near the site of stimulation, while large movements require more extensive bilateral muscle activation. Together, our results suggest that movement of the tail during the NWR is dominated by a co-contraction synergy.

Disclosures: C.L. Cleland: None. **A.C. Kahle:** None. **L. Harris:** None. **E. Dickson:** None. **A. Peroe:** None. **T. Tolley:** None. **L. Jagannathan:** None. **A. Adawi:** None.

Poster

P571. Reflexes and Reflex Modulation II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P571.09

Topic: E.06. Posture and Gait

Title: Postural movements accompany the heat-evoked tail and limb nociceptive withdrawal response in unrestrained rats

Authors: *M. PAVLICHENKO, S. MAVI, M. PEGELOW, C. L. CLELAND;
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Abstract: The nociceptive withdrawal response (NWR) is commonly used to elucidate movement strategies employed by the CNS. NWR studies typically use animal restraint, most commonly a tube to study tail movement, or a box to study limb movement. However, it is possible that restraints change behavior in comparison to rats that roam freely, potentially by obscuring whole body movements associated with the NWR of the tail and foot. Furthermore, restraint in a tube could also change the NWR tail movement itself, leading to different latencies, magnitudes, and directions of response. Thus, our goal was to quantify differences in the heat evoked NWR in the tail and limbs of rats that were either "restrained" or "free range". Rats (n=15) were restrained in clear tubes or boxes, or allowed to roam freely over a 3'x3' glass table. Foot and tail NWRs were elicited by noxious heat stimuli (980 nm laser diode) delivered to the lateral aspect of the tail at five rostral-caudal locations, or the plantar surface of each of the four feet. Low speed (60 fps) video was recorded from below the rat, or high-speed video (650 fps) was recorded from above. Low speed video data was collected to quantify whole body movements in free range trials, while high speed data was collected specifically to compare

restrained and free range tail movements. Heat stimulation of the tail and foot in free range rats evoked NWRs that were accompanied by short latency (133 ms) whole body movements. These movements were either a 180° rotation or movement in the forward direction, with individual rats evoking consistent responses across trials and days. Comparing high speed video of the tail NWR in restrained and free range rats indicated no significant difference in latency, magnitude, or direction of response. Together, our results indicate that whole body movement consistently accompanied free range tail and foot NWRs evoked by heat stimuli, either as a 180° rotation or as movement forward. Other known movements that consistently accompany NWRs are the crossed extension reflex, head turn and front paw stepping, which suggest that the function of the NWR should be understood in the context of a broader constellation of responses. Interestingly, rats that turn 180° do so consistently across trials and days, while rats that move forward are also similarly consistent. Finally, quantification of the tail NWR in restrained and free range rats showed no significant differences in latency, magnitude, or direction, which validates the use of restraint in tail NWR studies.

Disclosures: M. Pavlichenko: None. **S. Mavi:** None. **M. Pegelow:** None. **C.L. Cleland:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.01

Topic: F.02. Behavioral Neuroendocrinology

Support: UMASS FRG

Title: Chemogenetic activation of the medial preoptic area ameliorates deficits in maternal behavior in a rat model of postpartum depression

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Abstract: Maternal behavior that is sensitive to the needs of young is essential for the healthy development and wellbeing of all mammals. Mothers who experience postpartum depression often exhibit deficits in maternal behavior. Our prior research suggests that the medial preoptic area (mPOA), a brain region critical for maternal behavior, plays a significant role in coupling appropriate caregiving with the needs of the young. The goal of the present study is to examine whether hypofunction of the maternal mPOA underlies depression-related parenting disturbances in new mothers. To accomplish this goal, we used the Wistar-Kyoto (WKY) rat model of depression and excitatory Gq-DREADD (Gq-coupled designer receptors exclusively activated by

designer drugs) to activate the mPOA of WKY mothers during interaction with pups. WKY mother rats display a depression-like phenotype, including deficient maternal behavior, relative to control Sprague-Dawley (SD) rats, reminiscent of those in human mothers with postpartum depression. Elevating the activity of the mPOA ameliorated deficits in maternal behavior of WKY mothers compared to vehicle-treated WKYs, to levels characteristic of control SD mothers. Other behaviors, including general exploration, self-grooming, and eating or drinking, were not affected by chemogenetic activation of the mPOA. Together, our results suggest that the mPOA plays a key role in coupling sensitive caregiving behaviors to resolve the needs of the young.

Disclosures: A. Anderson: None. **K. Pizarro-Colon:** None. **M. Pereira:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.02

Topic: F.02. Behavioral Neuroendocrinology

Support: R15-MH118692
R01-MH112768

Title: Alterations in corticotropin-releasing factor receptor type 1 in the preoptic area and hypothalamus in mice during the postpartum period

Authors: *D. G. ZULOAGA[1], R. M. DE GUZMAN[1], Z. J. ROSINGER[1], K. E. PARRA[1], N. J. JUSTICE[2], J. JACOBSKIND[1];
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Abstract: Corticotropin-releasing factor (CRF) signaling through CRF receptor 1 (CRFR1) regulates autonomic, endocrine, and behavioral responses to stress, as well as behavioral changes during the maternal period. Previous work in our lab reported higher levels of CRFR1 in female compared to male mice within the rostral anteroventral periventricular nucleus (AVPV/PeN), a brain region involved in maternal behaviors. In this study, we used CRFR1-GFP reporter mice to investigate whether the reproductive status (postpartum vs. nulliparous) of acutely stressed females affects levels of CRFR1 in the AVPV/PeN and other regions involved in maternal functions. Compared to nulliparous, postpartum day 14 females showed increased AVPV/PeN CRFR1-GFP immunoreactivity and an elevated number of restraint stress-activated AVPV/PeN CRFR1 cells as assessed by immunohistochemical co-localization of CRFR1-GFP and phosphorylated CREB (pCREB). The medial preoptic area (MPOA) and paraventricular hypothalamus (PVN) of postpartum mice showed modest decreases in CRFR1-GFP

immunoreactivity, while increased CRFR1-GFP/pCREB co-expressing cells were found in the PVN following restraint stress relative to nulliparous mice. Tyrosine hydroxylase (TH) and CRFR1-GFP co-localization was also assessed in the AVPV/PeN and other regions and revealed a decrease in co-localized neurons in the AVPV/PeN and ventral tegmental area of postpartum mice. Corticosterone analysis of restrained mice revealed blunted peak, but elevated recovery, levels in postpartum compared to nulliparous mice. Finally, we investigated projection patterns of AVPV/PeN CRFR1 neurons using female CRFR1-Cre mice and revealed dense efferent projections to several preoptic, hypothalamic, and hindbrain regions known to control stress-associated and maternal functions. Together, these findings contribute to our understanding of the neurobiology that might underlie changes in stress-related functions during the postpartum period.

Disclosures: D.G. Zuloaga: None. **R.M. De Guzman:** None. **Z.J. Rosinger:** None. **K.E. Parra:** None. **N.J. Justice:** None. **J. Jacobskind:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.03

Topic: F.02. Behavioral Neuroendocrinology

Support: R15-MH118692
R01-MH112768

Title: Dynamic changes in expression of corticotropin-releasing factor receptor 1 within oxytocin neurons of postpartum mice

Authors: *K. E. PARRA[1], R. M. DE GUZMAN[1], Z. J. ROSINGER[1], J. S. JACOBSKIND[1], K. A. RYBKA[1], M. SHARIF[1], L. UGARTEMENDIA UGALDE[2], N. J. JUSTICE[2], D. G. ZULOAGA[1];
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Abstract: The postpartum period is characterized in part by a hypoactive stress response. This persistent state of resilience that mothers experience is adaptive in helping to maintain maternal care in the face of stressors and can likely be accounted for by changes in the hypothalamic-pituitary-adrenal (HPA) axis after parturition. In particular, using two transgenic mouse lines (CRFR1-GFP and CRFR1-Cre-tdTom), we found that corticotropin-releasing factor receptor 1 (CRFR1) uniquely appears in oxytocin neurons in the PVN and SON in postpartum mice. Male mice and reproductively naïve female mice do not possess this co-localization of CRFR1 and oxytocin neurons, and CRFR1 itself is entirely absent from the SON of nulliparous females. Furthermore, no co-localization of CRFR1 with PVN vasopressin neurons were found in any

mice. CRFR1/OT co-localization increases gradually in the PVN and SON throughout the postpartum period and peaks around postpartum day (PD) 21, and persists until at least PD 28. Using c-Fos immunohistochemistry, we were able to determine that these CRFR1/OT neurons are activated by acute restraint stress, indicating that these neurons might be contributing to the difference in stress response observed in postpartum mice as measured through lower CORT concentrations in postpartum relative to nulliparous mice following the stressor. To further illustrate the functional implications of this co-expression of CRFR1 in oxytocin neurons, we used in vitro electrophysiology in magnocellular PVN neurons to detect an increase in firing rate in presumed oxytocin neurons in response to CRF bath application to the region. This research provides insights into a potentially adaptive mechanism that occurs in postpartum females which might serve to increase stress resilience and facilitate maternal behavior under conditions of stress. The functional role of this neuroadaptation will be tested in future experiments.

Disclosures: K.E. Parra: None. **R.M. De Guzman:** None. **Z.J. Rosinger:** None. **J.S. Jacobskind:** None. **K.A. Rybka:** None. **M. Sharif:** None. **L. Ugartemendia Ugalde:** None. **N.J. Justice:** None. **D.G. Zuloaga:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.04

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R03HD097085

Title: Female reproduction affects CRF receptor expression in the midbrain dorsal raphe

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Abstract: The transition to motherhood involves neurobiological changes that allow for the display of caregiving behaviors. In both women and laboratory rodents, two hallmarks of motherhood that facilitate caregiving are reduced anxiety and stress responsiveness. This is partially controlled by the neuropeptide, CRF, acting in many brain sites. In male rodents, CRF influences affective behaviors and stress responsiveness by influencing serotonin originating in the midbrain dorsal raphe (DR). Previous work from our lab has shown that DR serotonergic output is essential for female postpartum affective and caregiving behaviors. To uncover the neuromechanisms of the reduced anxiety and stress responsiveness seen in the postpartum period, the current study aimed to quantify CRF receptor (CRFR) expression within the female rat DR across reproduction. In Experiment 1, levels of CRFR1 and CRFR2 mRNA within the

DR of virgin, mid-pregnant, recently parturient, and postpartum day 7 females were assessed using qPCR. More CRFR2 mRNA was found in the recently parturient and postpartum day 7 females compared to virgins, while less CRFR1 mRNA was found in recently parturient females compared to virgins. Experiment 2 using dual-label fluorescent *in situ* hybridization (RNAscope, ACDBio) is determining if this CRFR2 increase and CRFR1 decrease occurs on serotonin and/or GABA neurons. Colocalization of CRFR1 and CRFR2 with GAD_{65} and TPH2 in all 5 subregions of the DR is being assessed within these same groups. Virgin males are included to identify possible sex differences. These results will further uncover the CRFR-related mechanisms driving the reduction in postpartum anxiety and stress responsiveness. They also have implications for when this change does not occur, such as in cases of postpartum depression and/or anxiety, which affects 15-20% of peripartum women worldwide. Further understanding of the neurochemical mechanisms driving postpartum affective behaviors will inform the generation of new treatments for these disorders, and improve the lives of millions of women and their children.

Disclosures: T.A. Meinhardt: None. **Z.A. Grieb:** None. **J.S. Lonstein:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.05

Topic: F.02. Behavioral Neuroendocrinology

Support: Keith Rayner Memorial Graduate Student Research Award, awarded to Sarah Winokur
UMass Amherst Graduate School Dissertation Research Grant, awarded to Sarah Winokur

Title: Altered early postpartum period activity of estradiol, progesterone, and oxytocin is associated with the severity of depression-related maternal behavior deficits in Wistar-Kyoto rats

Authors: *S. B. WINOKUR, K. PIZARRO-COLON, P. MUDDASANI, K. TAKINAMI, O. YILDIRIM, V. LEE, N. SORIAL, M. PEREIRA;
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Abstract: Maternal caregiving behaviors in the early postpartum period are at a deficit in mothers who experience depression during the postpartum period. The risk of developing maternal behavior dysfunction associated with postpartum depression (PPD) has previously been linked with altered gestational levels of estradiol and progesterone, as well as decreased medial preoptic area (mPOA) oxytocinergic activity in the early postpartum period. It is currently unclear whether estradiol and progesterone signaling actively contribute to ongoing depression-

related maternal behavior deficits. Estradiol, via the estradiol receptor alpha (ERα), stimulates the release of oxytocin from the hypothalamus. Thus, disrupted early postpartum estradiol activity could actively disrupt oxytocinergic synthesis and action, leading to depression-related maternal behavior deficits. Considering that PPD is a highly prevalent disorder that impacts maternal health and well-being as well as child socio-emotional development, determining the role of postpartum estradiol, progesterone, and oxytocin in ongoing maternal behavior is critical to bettering diagnostic measures and treatments for depression-related disturbances in parenting. The goal of the current study was to investigate the association between maternal behavior and estradiol, progesterone, and oxytocin signaling. To this aim, we used Wistar-Kyoto (WKY) rats, who display a depression-like phenotype and deficient maternal caregiving behaviors relative to control Sprague-Dawley (SD) rats, which recapitulates many features of depression in human mothers. Estradiol and progesterone serum concentrations were measured in early postpartum SD and WKY mothers via ELISAs, ERα and oxytocin receptor levels were evaluated via qPCR, and ERα, oxytocin, and cFOS expression was measured via immunohistochemistry (IHC) in the maternal mPOA. Together, our data suggest that increased estradiol and progesterone activity in the mPOA mediates the severity of depression-related maternal behavior deficits. Ongoing analyses aim to evaluate this process in other maternal mesocorticolimbic brain regions to begin piecing together a functional circuit that underlies depression-related maternal behavior deficits.

Disclosures: S.B. Winokur: None. **K. Pizarro-Colon:** None. **P. Muddasani:** None. **K. Takinami:** None. **O. Yildirim:** None. **V. Lee:** None. **N. Sorial:** None. **M. Pereira:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.06

Topic: F.02. Behavioral Neuroendocrinology

Support: NARSAD

Title: The impact of chronic stress on perifornical urocortin-3 neurons in virgin and lactating female parenting

Authors: *B. ABDELMESIH, R. ANDERSON, A. AUTRY;
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Abstract: Parenting is a set of behaviors aimed at providing protection and care for young during early development and in some species, including mammals, is necessary for survival. Infant directed behaviors are complex, sexually dimorphic behaviors governed by neural circuits that are still poorly understood. Although laboratory female mice are typically parental towards infants, stress can lead to adverse behaviors such as infant neglect or even attack. Chronic stress

has been shown to be a prominent risk factor in developing postpartum mood disorders but its impact on genetically defined neural populations relevant to parenting is not well understood. Recently, our lab uncovered a genetically defined population of neurons that lead to negative infant-directed behaviors, urocortin-3 neurons in the perifornical area of the hypothalamus ($PeFA^{ucn3}$). When these neurons are inhibited in virgin females using a pharmacogenetic method (designer receptors exclusively activated by designer drugs [DREADD]), females retrieved pups to the nest faster. We then sought to interrogate the effects of chronic stress on parenting behavior and the activation levels of $PeFA^{ucn3}$ in both virgin and lactating females. Using two different models of chronic stress, we observed differences in how chronic stress affects both parental behaviors and $PeFA^{ucn3}$ activation between virgin and lactating females. We then successfully used inhibitory DREADDs on $PeFA^{ucn3}$ to ameliorate stress-induced deficits in parenting. Our findings help elucidate how chronic stress impacts a key neural population involved in parenting and suggest that activation levels are altered to protect against the adverse effects of stress in motherhood.

Disclosures: B. Abdelmesih: None. **R. Anderson:** None. **A. Autry:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.07

Topic: F.02. Behavioral Neuroendocrinology

Support: Funds were provided for medical and biological research by the State of Washington Initiative Measure No. 171

Title: Effects of cannabis use during pregnancy on maternal care and anxiety-like behavior in rats

Authors: *A. M. BROWN[1], A. N. MALENA[1], H. V. WEIMAR[1], H. R. WRIGHT[1], D. E. GINDER[2], R. J. MCLAUGHLIN[1,2];
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Abstract: The use of cannabis during pregnancy is a growing public health concern. Unfortunately, as the cannabis industry has been rapidly expanding, research on the effects of cannabis use during pregnancy remains limited and the effects of maternal cannabis use on both the mother and offspring are still largely unknown. Human studies indicate that women who use cannabis during pregnancy are more likely to experience post-partum symptoms of depression, but a causal relationship has not yet been systematically investigated. To address this gap in knowledge, we implemented a novel model of cannabis vapor self-administration in pregnant rat

dams to examine the extent to which maternal cannabis use affects maternal care and anxiety-like behavior during the post-partum period. Female Sprague Dawley rats were trained to self-administer either d^9 tetrahydrocannabinol (THC)-dominant cannabis vapor (69.9% THC; 150 mg/mL) or vehicle vapor on a fixed-ratio-1 schedule during one-hour sessions twice daily throughout mating and gestation. Maternal care behavior was video monitored during the first postnatal week and analyzed using pose estimation and machine learning approaches. After weaning, dams were subjected to a battery of behavioral assays designed to measure stress coping and anxiety-like behaviors, and plasma concentrations of circulating corticosterone were measured following acute swim stress to determine whether there are long-term changes in behavioral and neuroendocrine stress reactivity following maternal cannabis use. Results indicate that cannabis self-administering dams showed greater discrimination for the vapor-associated operandum and received significantly more vapor deliveries than VEH self-administering dams. Preliminary data indicate no significant difference in elevated plus maze behavior in cannabis-exposed dams relative to vehicle-exposed dams. Other endpoints are currently being analyzed, but we hypothesize that maternal cannabis use will decrease the frequency of maternal care events and the time spent engaging in pup-directed behavior. Additionally, we predict that maternal cannabis use will increase time spent immobile in the forced swim test and augment swim stress-induced corticosterone secretion compared to dams that had self-administered vehicle vapor. Ultimately, we hope that adopting this approach will generate more translationally relevant preclinical data that can be used to inform pregnant mothers of potential effects of consuming cannabis on their postpartum mental health, as well as the potential effects in their offspring.

Disclosures: A.M. Brown: None. **A.N. Malena:** None. **H.V. Weimar:** None. **H.R. Wright:** None. **D.E. Ginder:** None. **R.J. McLaughlin:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.08

Topic: F.02. Behavioral Neuroendocrinology

Support: ReBUILDetroit Bridge Grant

Title: Comparing the impact of morphine and buprenorphine on maternal care and behavior in a translational rodent model of opioid exposure during pregnancy

Authors: C. M. WALLIN, S. R. NEOLE, L. M. RICHARDSON, N. KULAGLIC, A. M. MYERS, S. E. BOWEN, *S. BRUMMELTE;
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Abstract: The opioid epidemic has resulted in many opioid-dependent pregnant women being pharmacologically treated with opioid maintenance therapies (e.g., buprenorphine or methadone) to combat the negative effects of misused opioids on the mother and developing offspring. Clinically, buprenorphine produces preferable outcomes for exposed infants compared to methadone or misused opioids. However, the behavioral and neurological effects of buprenorphine on the maternal brain and maternal caregiving behaviors are not well understood. Given differences in buprenorphine's mechanism of action (partial mu-agonist, kappa antagonist) and that of morphine (full mu-agonist), exposure to these drugs during pregnancy may have distinct effects on neurological networks critical in the transition to motherhood. We implemented a translational rodent model to mimic chronic opioid (mis)use (morphine exposure, 2-6mg/kg/day s.c.) or maintenance opioid drug use (buprenorphine exposure, 1mg/kg/day s.c.) to investigate the behavioral and neurochemical consequences of gestational opioid exposure on dams and their offspring. Female rats (N=50) were mated with drug naive males (N=10) in house. Opioid or saline administration to females began prior to pregnancy and was either continued until postpartum day 2 or discontinued shortly before parturition on gestational day 19. Dams were evaluated for maternal behaviors through meticulous observations of licking and grooming, nursing, avoidance, nesting behaviors and other pup directed or non-pup directed behaviors. Further, dams were challenged using several behavioral tests including a pup retrieval test (motivation assessment), a cricket hunting task (behavioral selection assessment), and a two-chamber preference test (assessment of preference for pup odor). Our preliminary findings indicate that both continued and discontinued buprenorphine exposure resulted in more maternal care deficits and pup avoidance, increased postpartum pup mortality, and maternal deficiencies in both the pup retrieval task and hunting task, as compared to dams in our saline-control group. On the other hand, both continued and discontinued morphine exposure seemed to result in fewer deficits in maternal behavior as compared to buprenorphine. Future analyses will evaluate the brains collected from opioid-exposed dams on postpartum day 2 for neurological changes. More research is essential to understand how buprenorphine interacts with the maternal brain network during the transition to motherhood to help avoid possible negative consequences on maternal care, the maternal brain and compounded effects on opioid-exposed offspring.

Disclosures: C.M. Wallin: None. **S.R. Neole:** None. **L.M. Richardson:** None. **N. Kulaglic:** None. **A.M. Myers:** None. **S.E. Bowen:** None. **S. Brummelte:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.09

Topic: F.02. Behavioral Neuroendocrinology

Support: ReBUILDetroit Bridge Award

Betty Neitzel Research Award

Title: A translational rodent model of gestational buprenorphine or morphine exposure: offspring outcomes

Authors: *A. M. MYERS, N. KULAGLIC, S. R. NEOLE, L. M. RICHARDSON, C. M. WALLIN, S. E. BOWEN, S. BRUMMELTE;
Dept. of Psychology, Wayne State Univ., Detroit, MI

Abstract: The recent opioid epidemic and escalation of opioid use during pregnancy have resulted in a drastic increase in gestational exposure to opioids. Opioid-dependent women are typically given opioid maintenance therapies (i.e., buprenorphine or methadone) to mitigate harmful effects on the fetus. However, the consequences of exposure to synthetic opioids such as buprenorphine during gestation on fetal outcome and brain development are poorly understood. Our translational rodent model aims to characterize neonatal mortality and morbidity after continued or discontinued gestational opioid exposure. Female rats received either morphine (2-6mg/kg twice daily, s.c.; to mimic opioid use disorder) or buprenorphine (1mg/kg s.c.; to mimic opioid maintenance therapy) from preconception until late gestation ('discontinued' on gestational day (GD) 19, mimicking withdrawal before parturition) or until postnatal day 2 ('continued'). Pups were either sacrificed on postnatal day 2 or fostered to a naïve foster dam and allowed to reach young adulthood for behavioral testing. Buprenorphine resulted in higher pup mortality, reduced body weight, and increased neonatal withdrawal symptoms compared to controls and morphine-exposed pups. Somewhat surprisingly, discontinuation of buprenorphine on GD 19 did not appear to rescue these effects. Preliminary behavioral data from young adult offspring suggest that gestational opioid exposure did not result in changes to anxiety-like behavior (elevated zero maze) or pain sensitivity (hot-plate test). This suggests that fostering opioid-exposed offspring may potentially rescue or protect them from negative effects of gestational opioid exposure or opioid-induced changes in maternal care. Offspring brains collected at postnatal day 2 will be analyzed to determine the effects of gestational opioid exposure on brain-derived neurotrophic factor (BDNF) concentration in the hippocampus and prefrontal cortex as a measure of neurodevelopment. More research is needed to understand how buprenorphine affects pregnancy outcomes and subsequent offspring well-being in order to avoid negative consequences in human mothers undergoing opioid maintenance treatment.

Disclosures: A.M. Myers: None. **N. Kulaglic:** None. **S.R. Neole:** None. **L.M. Richardson:** None. **C.M. Wallin:** None. **S.E. Bowen:** None. **S. Brummelte:** None.

Poster

P593. Female Reproductive Effects on Brain and Behavior

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P593.10

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF PRFB 2010906

Title: Sex, aggression and parental care: The neural and transcriptomic underpinnings of context dependent behavior

Authors: *R. DEANGELIS[1], M. BALLARD[1], H. A. HOFMANN[2];
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Abstract: Social behavior is inherently complex as individuals must integrate dynamic environmental cues within their current social context to display adaptive behavior. Pair bonding and parental care each comprise suites of behavioral displays with the motivational state of either promoting reproductive opportunities or increasing offspring survival. Here, we ask whether behaviors with shared motivational states (e.g., offspring care vs. offspring defense) share similar neural activity patterns and single-cell transcriptomes in dopaminergic reward regions of the brain. Conversely, neural substrates underlying behaviors similar in display (e.g., offspring defense vs. mate defense), but with distinct motivational states, may share cellular and transcriptomic similarity in brain areas sensitive to environmental information. The monogamous convict cichlid (*Amatitlania nigrofasciata*) is an ideal model system, as males and females readily form pair bonds, which they reinforce through affiliative displays, while also aggressively guarding mates against conspecific rivals. Additionally, both sexes jointly provision their offspring and vigorously defend them against predators. Hence, both pair bonding and parental care contain stereotypical elements of aggression and affiliation. Using immunohistochemical detection of the phospho-S6 ribosomal protein (pS6), we first identified patterns of neural activity throughout the vertebrate Social Decision-Making Network (SDMN) and the activity of dopaminergic, oxytocin, and vasopressin neurons in key nodes of the SDMN. We then used single nuclei RNA sequencing (snRNA-seq) of the preoptic area to identify (i) cell types (ii) the transcriptional response of behaviorally active cells and (iii) gene co-expression modules associated with either distinct motivational states and/or behavioral displays. The integration of both brain wide patterns of neural activity, the distribution and activation of oxytocin, vasopressin, and dopamine neurons, as well as the transcriptional response of POA cells will broaden our understanding of how context-dependent social behavior is coordinated within the brain.

Disclosures: R. DeAngelis: None. **M. Ballard:** None. **H.A. Hofmann:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Fetal and neonatal iron deficiency disrupts inner retinal vasculature development

Authors: N. C. A. R. RIBEIRO[1], C. LEWIS[2], B. K. HANSON[3], C. A. SERFATY[1], *G. W. ANDERSON[3];
[1]Univ. Federal Fluminense, Niterói, Brazil; [2]Univ. of Minnesota Duluth, Duluth, MN; [3]Univ. of Minnesota, Duluth, MN

Abstract: The retina is the most metabolically active tissue in the body. To meet this high metabolic demand, this tissue has two vascular systems: one external (the choroidal vasculature) and one internal (the inner retina vasculature). Iron (Fe) is required for oxygen transport and is also involved in several enzymatic and metabolic reactions in the retina. Fe deficiency (FeD) is associated with delayed latencies on visual evoked potentials in children. In this work, we evaluated how FeD during the fetal and neonatal period affects retinal development. Sprague-Dawley pregnant rats were fed an Fe-deficient diet on embryonic day 2 (E2). Control (CT) dams were fed an Fe-sufficient diet and both groups drank deionized water. Tissues were harvested from rat pups on postnatal days (P) 7, 14 and 21. FeD significantly lowered blood hemoglobin levels in FeD compared to control pups at P7 (FeD 80.37 mg/ml ± 4.3 n:12; CT 131.3 mg/ml ± 6.0 n:14 $p< 0.05$) and at P14 (FeD 67.18 mg/ml ± 2.7 n:18; CT 119.1 mg/ml ±5.1 n:17 $p<0.05$). RNA sequencing (RNA-seq) was performed on harvested P14 retinal tissue (6 CT and 6 FeD) to investigate FeD impact on the transcriptome. We identified 26 differentially expressed genes including *Lgr1, CxCL12, Apln, Adgr6, Flt1, PFKB3, Adgrf5 and Lats2* (angiogenesis and vasculogenesis associated genes), *Vwf* and *Nrgn* (endothelial cell associated genes), and *C1qa,* a gene expressed in retinal microglial cells and involved in retinal angiogenesis and retinal degeneration. We further analyzed the impact of FeD on blood vessel formation in the developing retina through immunohistochemical approaches. We found increased arterial branch points/mm vessel length in the superficial layer of vessels in FeD at P7 (FeD 39 branch points ± 1.5 n:6; CT 30.4 branch points ± 2.5 n:5 $p<0.05$) and P14 (FeD 25.6 branch points ± 2.3 n: 7; CT 33.7 branch points ± 1.8 n:6 $p<0.05$) demonstrating increased retinal vascular density in FeD. The arteriole width also increased in FeD (FeD 39.7 micrometers ± 1.2 n:8; CT 30.3 micrometers ± 1.0 n:8 $p<0.05$) at P14. Confocal Z-stack images showed an increase in the number of microglia cells in the deep layer of blood vessels (FeD 15.6 cells/area ± 0.1 n:5; CT 11 cells/area ± 1.3 n:6 $p<0.05$) at P14. These results show that developmental FeD impacts retinal blood vessel formation. These impacts are likely driven through responses to tissue hypoxia and may contribute to perturbation in visual function.

Disclosures: N.C.A.R. Ribeiro: None. **C. Lewis:** None. **B.K. Hanson:** None. **C.A. Serfaty:** None. **G.W. Anderson:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS103844
NIH Grant T32GM008349
NSF Grant 1747503

Title: Notch3 directs differentiation of brain mural cells from human pluripotent stem cell-derived neural crest

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Abstract: Objective and rationale: Brain pericytes and vascular smooth muscle cells, collectively termed mural cells, regulate development and function of the blood-brain barrier (BBB) and control brain blood flow. Unlike the mesoderm-derived mural cells of other organs, forebrain mural cells are derived from the neural crest. Molecular signals controlling this differentiation process are poorly understood, with loss of function experiments suggesting roles for TGF-β, PDGF, and Notch signaling. Furthermore, existing *in vitro* models of human brain mural cells derived both from primary cultures and human pluripotent stem cells (hPSCs) have markedly reduced expression of key mural cell genes, including *NOTCH3,* compared to *in vivo* samples, and require serum-supplemented culture media. Thus, we sought to determine whether activation of Notch3 in hPSC-derived neural crest could direct the differentiation of brain mural cells with an improved transcriptional profile. **Methods and results:** We generated a bicistronic lentiviral vector encoding the human Notch3 intracellular domain and green fluorescent protein (N3ICD-GFP). We transduced p75-NGFR$^+$ HNK-1$^+$ neural crest cells derived from hPSCs with N3ICD-GFP lentivirus or GFP-only lentivirus in serum-free E6 medium. Compared to GFP-transduced neural crest, N3ICD-GFP-transduced cells exhibited upregulated expression of the mural cell transcripts *PDGFRB, RGS5*, *FOXS1*, *TBX2*, and *HEYL*. In contrast, transduction of TBX2-GFP did not produce such an effect. In N3ICD-GFP-transduced cells, we observed increased protein-level expression of PDGFRβ and both full-length and cleaved Notch3. We additionally evaluated the ability of the resulting cells to self-assemble with endothelial cells (ECs) in a cord-formation assay and to induce BBB properties in ECs. **Conclusions:** Our data suggest that activation of Notch3 signaling is sufficient to direct differentiation of neural crest to brain mural cells, and that multiple brain mural cell-associated transcription factors are downstream of Notch3 signaling. We also establish a new, serum-free differentiation protocol for generation of hPSC-derived brain mural cells for downstream *in vitro* modeling applications.

Disclosures: B.D. Gastfriend: None. **M.E. Snyder:** None. **R. Daneman:** None. **S.P. Palecek:** None. **E.V. Shusta:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: PJT-153009
RGPIN-2017-05184

Title: Anatomical organization of the rat subfornical organ

Authors: *A.-I. HICKS[1], S. KOBRINSKY[2], S. ZHOU[1], J. YANG[3], M. PRAGER-KHOUTORSKY[4];
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Abstract: The subfornical organ (SFO) is a sensory circumventricular organ located along the anterodorsal wall of the third ventricle. SFO lacks a complete blood-brain barrier (BBB), and thus peripherally-circulating factors can penetrate the SFO parenchyma. These signals are detected by local neurons providing the brain with information from the periphery to mediate central responses to humoral signals and physiological stressors. Circumventricular organs are characterized by the presence of unique populations of non-neuronal cells, such as tanycytes and fenestrated endothelium. However, how these populations are organized within the SFO is not well understood. In this study, we used histological techniques to analyze the anatomical organization of the rat SFO and examined the distribution of neurons, fenestrated and non-fenestrated vasculature, tanycytes, ependymocytes, glia cells, and pericytes within its confines. Our data show that the shell of SFO contains non-fenestrated vasculature, while fenestrated capillaries are restricted to the medial-posterior core region of the SFO and associated with a higher BBB permeability. In contrast to non-fenestrated vessels, fenestrated capillaries are encased in a scaffold created by pericytes and embedded in a network of tanycytic processes. Analysis of c-Fos expression following systemic injections of angiotensin II or hypertonic NaCl reveals distinct neuronal populations responding to these stimuli. Hypertonic NaCl activates ~13% of SFO neurons located in the shell. Angiotensin II-sensitive neurons represent ~31% of SFO neurons and their location varies between sexes. Our study provides a comprehensive description of the organization of diverse cellular elements within the SFO, facilitating future investigations in this important brain area.

Disclosures: A. Hicks: None. **S. Kobrinsky:** None. **S. Zhou:** None. **J. Yang:** None. **M. Prager-Khoutorsky:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.04

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS108407

Title: Regional Heterogeneity and Vulnerability of Pericyte Populations in the Mouse Brain During Normal and Pathological Aging

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Abstract: Aging is often complicated and accelerated by vascular and neurodegenerative diseases, such as Alzheimer's disease (AD). Increasing evidence indicates that vascular pathology, such as pericyte loss, precedes neuronal degeneration, and contributes to the aging progression and severity of AD. Specifically, recent studies have implicated pericyte dysfunction in both normal aging and neurodegenerative diseases. Pericytes are a mural cell type that primarily encapsulate the microvasculature, playing a critical role to maintain the integrity of the blood brain barrier. Despite this significance, questions remain as to whether there is loss of pericytes in normal aging and how this relates to the role of pericytes in AD. Here, we sought to study how pericyte populations across the whole mouse brain are impacted by aging as well as β-amyloid (Aβ) accumulation, since this is implicated in AD. We used transgenic reporter mice (PDGFRβ-Cre crossed with Ai14 mice) to label vascular mural cells, including pericytes, and compared 2-month-old adult mice with aged 18-month-old mice. We further crossed PDGFRβ-Ai14 mice with the 5xFAD model of Familial AD, as a model of aggressive and accelerated Aβ accumulation, to study how this insult may affect pericyte populations in the brain. These mice were studied at the symptomatic stage for this mouse model, which is at 8 months of age. We used serial two-photon tomography to image the entire mouse brain at cellular resolution and developed computational pipelines to selectively detect and map the distribution of capillary pericytes in a reference brain. Interestingly, we found that normal aging is associated with reduced pericyte density in regions of the basal forebrain at 18 months, particularly the magnocellular nucleus, which is often implicated in AD, as well as layer 6 across regions of the isocortex. Moreover, we identified that accumulation of Aβ in the 5xFAD model reduced pericyte density in several sensory regions of the thalamus and brainstem, as well as the retrosplenial cortex. Our data suggests that normal aging and aging in the setting of Aβ accumulation confer differing vulnerabilities of pericyte populations in multiple regions of the mouse brain.

Disclosures: H. Bennett: None. **Y. Wu:** None. **Y. Kim:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.05

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS R01NS092388 (RJN)
NIGMS 5U54GM104942-03

Title: Dim light at night exposure alters hippocampal vascular structure

Authors: J. R. BUMGARNER[1], W. H. WALKER II[1], O. H. MELÉNDEZ-FERNÁNDEZ[1], J. A. LIU[1], J. C. WALTON[1], A. C. DEVRIES[2], ***R. J. NELSON**[1];
[1]Neurosci., [2]Medicine; Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Artificial light at night (LAN) is a pervasive facet of modern life. Because circadian rhythms rely on consistently timed environmental cues to maintain internal and external synchrony, the ubiquity of LAN poses a serious challenge to health. LAN exposure leads to numerous negative physiological consequences, including impaired metabolic, endocrine, and immune function. LAN also increases the risk for diseases such as obesity, cancer, and cardiovascular disorders. Previous research has demonstrated that as few as 3-4 nights of LAN exposure can induce depressive-like responses, as well as alter cytokine and neurotrophic transcript expression in the hippocampus of rodents. Acute exposure to LAN also alters hippocampal expression of angiogenic signaling transcripts. Given the circadian regulation of the cardiovascular system, the consequences of LAN exposure on the structure of hippocampal vasculature were examined by exposing female and male CFW mice to 4-nights of LAN. We hypothesized that LAN exposure impairs the structural organization of the cerebrovasculature. Using corrosion casting and μCT imaging, whole mouse brain 3D vasculature datasets were generated, and the CA1, CA3, and dentate gyrus of the hippocampi were segmented. LAN was observed to reduce hippocampal vascular volume and alter the distribution of small-diameter vessels in both the CA1 and CA3. These results indicate that circadian rhythm disruption can impair the structural organization of cerebrovasculature, highlighting the need to reduce LAN in clinical and other contexts.

Disclosures: J.R. Bumgarner: None. **W.H. Walker II:** None. **O.H. Meléndez-Fernández:** None. **J.A. Liu:** None. **J.C. Walton:** None. **A.C. DeVries:** None. **R.J. Nelson:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.06

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01NS113912-01

Title: Modulation of iron release by endothelial cells by transferrin at the blood-brain barrier

Authors: *S. BARINGER, E. NEELY, K. PALSA, I. A. SIMPSON, J. R. CONNOR;
Penn State Col. of Med., Hershey, PA

Abstract: While iron plays an important role in many bodily functions, it is especially crucial to the highly metabolically active brain, acting as both an electron donor and acceptor in cellular metabolism. As such, maintaining a proper balance of brain iron levels is vital to cellular function. Excessive amounts of brain iron can lead to neurodegenerative diseases and inadequate amounts can lead to cognitive impairment and Restless Legs Syndrome. Regulation of iron uptake into the brain is required, however the mechanism of such regulation is largely unknown. Our lab has previously shown that brain iron uptake is mediated at the level of the endothelial cells (ECs) of the blood-brain barrier (BBB) and regulation is modulated by apo (iron poor) and holo (iron rich) transferrin (Tf) in the cerebrospinal fluid (CSF). **Here we aimed to address the mechanism by which apo- and holo-Tf control iron release from ECs.** Mini osmotic pumps were implanted into mice with a catheter inserted into the lateral ventricle. A steady-state infusion of sham (nothing), artificial CSF, 1 mg/ml of apo-Tf, or 1 mg/ml of holo-Tf occurred for 48 hours at which time the mice were injected intraperitoneally with radioactive ^{55}Fe-Tf or ^{55}Fe-H-ferritin (Fth1). After 24 hours, brains were collected and fractionated into microvessels and rest of whole brain. We found 1) infusion of apo-Tf increased ^{55}Fe-Tf uptake by 42% ($p<0.05$) and release into the brain by 40% ($p<0.05$) while holo-Tf did not, 2) there was a sex difference in the regulation of ^{55}Fe-Tf uptake, and 3) infusions did not have an effect of ^{55}Fe-Fth1 uptake. To investigate the mechanism of Tf's regulation on iron release from ECs, we employed human induced pluripotent stem (hiPSC)-derived ECs. ECs were loaded with ^{55}Fe on Transwell bi-chamber plates, and concentrations of apo- or holo-Tf ranging from 0.01 to 100 uM were placed in the basal chamber. By determining iron release from ECs at hourly intervals, the Km of apo-Tf iron release and Ki of holo-Tf iron release were calculated. In separate experiments, $FeCl_3$ loaded ECs were incubated with apo- or holo-Tf concentrations in the basal chamber. After 8 and 24 hours of incubation, the cells were collected and lysates were used in western blotting to probe for key iron regulatory protein expressions. We found that 0.1 uM holo-Tf decreased ferroportin (Fpn) expression by 50% ($p<0.05$). Collectively, these results demonstrate that apo- and holo-Tf modulate iron release from ECs through interactions with Fpn. These data establish the mechanism for regulation of iron uptake into the brain, demonstrate a sex-specific regulatory mechanism, and suggest the mechanism for regional regulation of brain iron uptake.

Disclosures: S. Baringer: None. **E. Neely:** None. **K. Palsa:** None. **I.A. Simpson:** None. **J.R. Connor:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.07

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH RO1NS117827
NIH RO1NS100459

Title: Cadasil pathology in a pericyte-specific inducible cre mouse

Authors: *A. M. NIKOLAKOPOULOU, Z. ZHAO, A. CHAKHOYAN, X. HE, B. V. ZLOKOVIC;
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Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare genetic disorder affecting the small blood vessels in the brain. CADASIL is associated with vascular accumulation of mutant Notch3 protein, dysregulation of cerebral vessels, and dementia. Pericytes, the vascular mural cells located within the basement membrane of blood microvessels that control BBB permeability, regulate cerebral blood flow (CBF) and participate in the clearance of brain toxic byproducts, express Notch3. Dysfunction of pericytes has been implicated in BBB breakdown, hypoperfusion and CBF reduction, inflammation, neuronal degeneration and cognitive impairment. Here, we report developing an inducible CADASIL (*ROSA26-LSL-Notch3$^{+/R169C}$*) pericyte-specific Cre (*Atp13a5-Cre$^{ERT2+/+}$)* mouse model (*Atp13a5-Cre$^{ERT2\ +/-}$; ROSA26-LSL-Notch3$^{+/R169C}$)*, where mutant Notch3 specifically accumulates in pericytes. This model will give us the advantage to specifically study the effects of aggregation of mutant Notch3 in brain pericytes and subsequent deficits of the blood brain barrier at any stage during lifespan and can be a useful tool in the study of neurodegeneration and development of therapeutics.

Disclosures: A.M. Nikolakopoulou: None. **Z. Zhao:** None. **A. Chakhoyan:** None. **X. He:** None. **B.V. Zlokovic:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.08

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant NS099292

Title: Transient Blood Brain Barrier Opening During Cortical Spreading Depression Results from Dynamic Reorganization of the Tight Junction Protein Claudin-5

Authors: *J. R. WAHL[1], A. VIVEK[1], S. PALOMINO[1], M. ALMUSLIM[1], K. E. COTTIER[2], E. LIKTOR-BUSA[1], J. M. STREICHER[1], T. P. DAVIS[1], T. W. VANDERAH[1], T. M. LARGENT-MILNES[1];
[1]Med. Pharmacol., Univ. of Arizona Col. of Med., Tucson, AZ; [2]Mol. Microbiology and Immunol., Johns Hopkins Univ., Baltimore, MD

Abstract: Cortical spreading depression (CSD) has long been suggested as a potential cause for pain experienced during migraine. CSD events trigger intracranial neurogenic inflammation and activation by releasing several substances into the extracellular space, including glutamate, K+, H+, and ATP. The resulting increase of K+ and glutamate in the extracellular space induces propagation of the CSD event by depolarizing surrounding neurons. CSD events are closely tied to potential pathophysiologic states of the blood brain barrier (BBB) and neurovascular unit (NVU). The BBB is composed of cerebral endothelial capillary cells tightly bound together by tight junction (TJ) proteins on the luminal side of vessel, and adherens junctions (AJ) proteins on abluminal side of vessel, creating a highly selective and permeable physical and biochemical barrier between peripheral circulation and the central nervous system (CNS). Dynamic alterations of the aforementioned tight junction proteins allow for a potentially pathologic alteration to the discrete regulation of substance influx and efflux to the brain present during normal homeostatic physiologic conditions at the BBB. One consequence of CSD is transient change in permeability of BBB of sufficient magnitude to allow small molecules (<400 g/mol) to pass from blood into the brain. Prior research has demonstrated total TJ protein expression remains unchanged during CSD, leading to the investigation of the underlying mechanisms occurring in these proteins responsible for the functional alterations leading to the observed openings in the BBB. My current studies therefore aim to elucidate these underlying mechanisms by investigating the hypothesis that reorganization of TJ proteins, not changes in overall expression, underscore the observed transient opening in the BBB during CSD events. Utilizing *in vitro* models of the BBB in addition to comparative studies utilizing animal, preclinical, and proteomic techniques, I aim to find a elucidate a physiologic link connecting CSD events in the cortex to the pathologic condition of migraine with aura.

Disclosures: J.R. Wahl: None. **A. Vivek:** None. **S. Palomino:** None. **M. Almuslim:** None. **K.E. Cottier:** None. **E. Liktor-Busa:** None. **J.M. Streicher:** None. **T.P. Davis:** None. **T.W. Vanderah:** None. **T.M. Largent-Milnes:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.09

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Alpha melanocyte stimulating hormone (α-MSH) alters brain endothelial function in ABCD1-deficient human brain endothelial cells via NUR77-dependant regulation of tight junction proteins.

Authors: *C. G. BERGEN[1], S. K. MCCARTHY[1], C. A. LUSK[1], P. L. MUSOLINO[2]; [1]Neurol., [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: X-linked adrenoleukodystrophy (ALD) is a neurologic disorder caused by mutations in ABCD1, a gene that encodes for the peroxisomal half-transporter adenosine triphosphate-binding cassette domain 1. This mutation causes an accumulation of very long chain fatty acids (VLCFAs) in body fluids and tissues. Most patients with ALD suffer from adrenal gland insufficiency and hyperpigmentation due to elevated levels of Adrenocorticotropic hormone (ACTH) and α-MSH. Using MetaCore genomic analysis, we determined ACTH and α-MSH melanocortin receptors (MCRs) pathway involved in regulation of endothelial tight junction (TJ) proteins. To assess whether loss of ABCD1 and adrenal insufficiency could contribute to the disruption and increased permeability of the blood brain barrier (BBB) seen in the cerebral demyelinating form of ALD we used siRNA to silence the gene in HBMECs and test molecular and functional changes after α-MSH stimulation via qPCR, Western Blot and transmigration. We found that α-MSH supplementation lead to decreased expression of NUR77 and Claudin-5, the predominant TJ in brain white matter endothelium. In turn, increased permeability to leukocytes was observed after α-MSH supplementation in ABCD1-deficient HBMECs compared to controls. This work presents for the first time a potential new therapeutic target that could restore endothelial TJ function and decrease BBB permeability to leukocytes to prevent cerebral demyelination in ALD. Further research of these pathways may uncover new links between melanocortin regulation, hormonal dysregulation and cerebral degeneration in ALD.

Disclosures: C.G. Bergen: None. **S.K. McCarthy:** None. **C.A. Lusk:** None. **P.L. Musolino:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.10

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS113912-01

Title: Exosomes are involved in iron transport from Human blood brain barrier endothelial cells and are modified by iron status.

Authors: *K. PALSA, S. L. BARINGER, I. A. SIMPSON, J. R. CONNOR;
Penn State Col. of Med., Hershey, PA

Abstract: Iron is essential for normal brain development, and its deficiency is associated with reduced cognitive performance. To enter the brain, transferrin (Tf) bound iron is taken up by endothelial cells (ECs) via transferrin receptor (TfR) at the luminal side. Iron is released within the ECs and can be egressed via Ferroportin (FPN)/Hephaestin (HEPH) dependent mechanism to the albuminal side. We previously demonstrated that ferritin heavy chain (HFT) also transport iron to the brain via endothelial cells, implying alternate iron transport mechanisms. Here-in we investigated role of exosomes in mediating the HFT or Tf mediated iron transport across the blood brain barrier (BBB) ECs. The study used ECs derived from human induced pluripotent stem cells (hiPSC) that are grown in bicameral chambers. hECs were labeled with ^{55}Fe from the apical side for a period of 24h. The cells were then treated with Desferroxamine (DFO, an iron chelator, 100µmol/L) or Ferric ammonium citrate (FAC, an iron source; 100µmol/L) or PBS or 1 mg/mL of human apo-Tf or 100 µg/mL HFT for 24 hours. Exosomes from the basal media were isolated by ultracentrifugation (120,000 *x g*), Exosomal content was confirmed by immunoblotting for CD81, an exosomal marker. We also measured the relative ^{55}Fe radioactivity, Tf, and HFT levels in the exosomal fraction as compared to the basal media supernatant. When cells were exposed to Tf or Hft, the ^{55}Fe activity in the exosomal fraction was 292% and 103% higher, respectively compared to the supernatant fraction. Tf and HFT were also released from the ECs in exosomes but only when the cells were exposed to apo-Tf and HFT treated cells not under control conditions. Moreover, the exosomal-associated Tf levels were higher when ECs were pre-treated with FAC and subsequently treated with apo-Tf versus pre-treatment with DFO. The number of exosomes were higher when cells were treated with FAC compared to DFO, as evidenced by higher CD81 density. These results indicate that iron transport across the BBB is mediated via the exosomal pathway and is modified by iron status of the hECs providing evidence for novel alternate mechanisms of iron transport into the brain.

Disclosures: K. Palsa: None. **S.L. Baringer:** None. **I.A. Simpson:** None. **J.R. Connor:** None.

Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.11

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Transferrin receptor expression (TfR) across species and age informs potential safety and efficacy of TfR-targeted therapies

Authors: *M. PIZZO, Y. CHENG, M. CALVERT, A. ARGUELLO, D. CHAN, K. CHEW, J.-H. CHOW, S. DEVOS, J. DUGAS, C. HA, K. HENNE, D. JOY, M. KARIOLIS, K. LECHTENBERG, S. LIANOGLOU, C. MAHON, T. SANDMANN, M. SIMON, E. THOMSEN, R. TONG, R. WELLS, M. DENNIS, A. ESTRADA, K. GADKAR, J. W. LEWCOCK, P. SANCHEZ, Y. J. Y. ZUCHERO, R. MEISNER, R. G. THORNE;
Denali Therapeut., South San Francisco, CA

Abstract: Understanding transferrin receptor (TfR) expression and distribution across species and different ages is critical for the translation of TfR-targeting therapeutics. However, few studies have carefully compared these aspects across the major species of importance in drug development of these biotherapeutics: mouse, macaque, and human. Here, we have utilized multiple methods to cross-validate TfR expression and distribution in normal tissues and with age in wild-type mice, chimeric TfRmu/hu knockin mice, cynomolgus monkeys, and humans. We compared TfR expression in the brain and peripheral organs using in situ hybridization, immunohistochemistry, and ultra-high multiplexed imaging to visualize expression and localization. RNA-seq and western blot was further used to understand overall level of expression across species. We observed a generally high level of agreement between species, with brain, bone marrow, placenta, and gastrointestinal tissues having among the highest levels of expression within certain cell types. Interestingly, TfR expression was high in Schwann cells of peripheral nerves from mouse and cynomolgus monkey samples, but TfR was mostly absent in human samples.

It is currently unclear whether TfR expression is altered with age and whether such changes, if present, may significantly impact the transport of TfR-targeting therapeutics into the brain. We therefore investigated the uptake of a monovalent mouse TfR-binding antibody in young and aged wild type mice. Immunohistochemistry of brain sections revealed high endothelial TfR protein expression in mice aged 3, 15, and 24 months. Importantly, brain concentrations of the TfR-binding antibody were unchanged with age. Cellular localization and level of expression in human brain was also evaluated between pediatric, adult, and aged samples.

Our characterization of TfR expression with age and across species will help inform the interpretation and translatability of studies evaluating the biodistribution, brain targeting, and efficacy of TfR-targeted therapeutics for neurodegenerative diseases, including antibody transport vehicles (Kariolis et al. Sci Transl Med 2020), enzyme transport vehicles (Ullman et al. Sci Transl Med 2020), and other TfR-binding architectures (Arguello et al. bioRxiv 2021).

Disclosures: **M. Pizzo:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **Y. Cheng:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **M. Calvert:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **A. Arguello:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

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Poster

P621. Blood Brain Barrier

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P621.12

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: University of Wisconsin-Madison startup grant (135 PRJ48TR)
University of Wisconsin-Madison School of Pharmacy (233 PRJ49HR)
University of Wisconsin Institute for Clinical and Translational Research Grant (NIH UL1TR000427 and KL2TR000428)
National Science Foundation (DGE-1256259)
University of Wisconsin-Madison Clinical Neuroengineering Training Program (NIH NRSA T32EB011424)
Michael J Fox Foundation for Parkinson's Research Training Pipeline grant (135 PRJ82AZ)
Wisconsin Alumni Research Foundation Accelerator Program (135 PRJ82AZ)

Title: Neonatal Fc receptor and Fc gamma receptor IIb expression at the brain-cerebrospinal fluid interfaces in the rat central nervous system

Authors: G. NEHRA[1], M. S. THOMSEN[2], S. BIRKELUND[3], G. GREENE[1], N. N. KUMAR[1], M. E. PIZZO[1], B. WILKEN-RESMAN[1], T. MOOS[2], ***R. G. THORNE**[1];
[1]Pharmaceut. Sci. Div., Univ. of Wisconsin-Madison, Madison, WI; [2]Lab. of Neurobiology, Dept. of Hlth. Sci. and Technol., [3]Lab. of Med. Mass Spectrometry, Dept. of Hlth. Sci. and Technol., Aalborg Univ., Aalborg, Denmark

Abstract: Immunoglobulin G (IgG)-based therapeutics are emerging as a promising treatment for central nervous system (CNS) disorders and characterizing distribution and efficacy of IgG in the central nervous system (CNS) will be critical for their successful translation. These characteristics are governed, in part, by the interaction between IgG and endogenous Fc receptors that bind to the crystallizable fragment (Fc region) of IgG immunoglobulins throughout the body. Suprisingly, the role of these interactions remains poorly understood at specialized fluidic interfaces in the CNS. Here, we investigated the expression and localization of two Fc receptors - (i) the neonatal Fc receptor (FcRn) that binds to IgG with high affinity at pH ~ 6.5, and (ii) the low affinity-binding ($K_d \sim 10^{-6}$ M) inhibitory Fc gamma receptor (FcγRIIb), at these interfaces in female Sprague-Dawley rats ($n = 4$). A similar immunohistochemical study was performed in rat and mouse brain-derived capillary endothelial cell cultures ($n = 4$) and glial cell cultures ($n = 4$). Cells from these primary cultures were also tested for mRNA expression of these receptors. We observed high FcRn and FcγRIIb immunoreactivity at the olfactory nerve layer, leptomeninges, glia limitans, perivascular spaces, ventricles, and choroid plexus in the rat brain. Cell immunoreactive for both FcRn and FcγRIIb predominantly included lymphatic endothelial cells in the meninges, perivascular macrophages, choroid plexus epithelial cells, and astrocytes. Diffuse immunoreactivity was also observed in neocortical neurons and astrocytes in the white matter tracts. In contrast, brain endothelial cells were immunoreactive for neither FcRn nor FcγRIIb in our study. In vitro immunofluorescence analysis in primary cell cultures of rat and mouse brain capillary endothelial cells further confirmed minimal FcRn and FcγRIIb immunoreactivity. Gene expression analysis further revealed high Fcgrt expression in isolated brain capillary endothelial cells for mice but not for rats. We hypothesize that high FcRn and FcγRIIb immunoreactivity in non-endothelial cells at these interfaces directly impacts IgG uptake and transport in the CNS by limiting IgG availability through weak/reversible binding at the perivascular and subarachnoid surfaces. Overall, our findings may potentially inform the engineering of antibody therapeutics for more efficient distribution within the CNS as well as provide new insights into the physiological factors influencing the distribution of endogenous IgG.

Disclosures: G. Nehra: None. **M.S. Thomsen:** None. **S. Birkelund:** None. **G. Greene:** None. **N.N. Kumar:** None. **M.E. Pizzo:** None. **B. Wilken-Resman:** None. **T. Moos:** None. **R.G. Thorne:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: T32NS096050

Title: Identifying Circuits That Underly Social Recognition Behavior

Authors: *M. RASHID;
Emory Univ. Neurosci. and Behavioral Biol., Atlanta, GA

Abstract: The ability to recognize familiar conspecifics from strangers is crucial for the survival of most mammalian species. In humans, an inability to recognize kin is associated with several types of dementia, including Alzheimer's disease. Despite the debilitating nature of social recognition deficits and the key role, social recognition plays in shaping animal behavior, the neural circuits underlying social recognition remain poorly understood. Recent evidence has determined the ventral hippocampus (vHPC) as necessary for social recognition. Of particular interest is the lateral septum since it is a major output of the vHPC and is a region that has been implicated in kin recognition. The exact contribution of vHPC projections to the lateral septum in allowing an animal to discriminate between familiar and novel conspecifics remains unknown. In this study, we used a combination of imaging and optogenetic methods to determine the role of the vHPC-LS projections in mediating social recognition. To test if vHPC-LS projections played a causal role in allowing mice to discriminate novel from familiar animals, we inhibited vHPC-LS neurons in a spatially specific manner in the proximity of either a novel or a familar mouse in a social discrimination assay. We found that inhibition of vHPC-LS neurons disrupted a mouse's ability to discriminate between a familiar and novel mouse. In contrast, control mice demonstrate an increased preference for the novel mouse. (ChR2 n=12 ;GFP n=7 , p=0 .037). To test if activating vHPC-LS neurons had an effect on social recognition, we first injected ChR2 bilaterally into vHPC and stimulated LS afferents while animals were engaged in a social discrimination task. Surprisingly, we found that activation of the anatomical subpopulation increased investigation around both a novel and familiar conspecific (ChR2 n=11 ; GFP n=8 , p=0 .032). In contrast, activating vHPC projections to the medial prefrontal cortex (mPFC) had no effect on social recognition behavior (ChR2 n=11 ; GFP n=8 , p=0 .041). These findings suggest that vHPC projections to the LS but not the PFC play a crucial role in social recognition. To further characterize the differences in neural dynamics of this population during social interactions with a novel versus a familiar mouse, we monitored the activity of the vHPC-LS using cellular-resolution imaging in freely moving mice in the same social discrimination task. Preliminary data shows that vHPC-LS neurons are active in the proximity of novel and familiar animals (n=2). By combining optogenetics and cellular resolution calcium imaging, this study provides the first insight into the role of vHPC-LS circuit in social recognition.

Disclosures: M. Rashid: None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH NIDDK Grant R01DK085721

Title: Fos expression in the basomedial amygdala after habituation to novel food and novel context

Authors: *Z. IRVING, E. GREINER, G. PETROVICH;
Boston Col., Boston, MA

Abstract: Responses to novel stimuli are both adaptive and variable. Environmental and physiological differences impact how individuals respond to novel food and novel feeding environments. Our prior work established that novel food and novel context inhibit food intake in food deprived male and female rats, and that there were sex differences during habituation, with females habituating slower to eating in a novel context. Furthermore, we recently found that the anterior basomedial nucleus of the amygdala (BMAa) is robustly recruited during the first exposure to novelty. To establish the neuronal substrates underlying habituation to novelty, here we examined recruitment of the BMAa after repeated exposure. Adult male and female Long Evans rats (n=8/group) were food deprived for 20h prior to consumption tests in a novel or familiar (their home cage) context. Rats were given novel Test Diet pellets across 4 habituation sessions and 1 final testing session. Rats were perfused 90 minutes after the start of the final testing session and brain tissue was immunohistochemically processed for Fos detection in the BMAa. Consumption patterns analyzed by grams eaten as percent of body weight showed that during the first habituation session, consumption differed by sex ($F(1,28)=6.978$, $p=0.013$) and context ($F(1,28)=12.84$, $p<0.01$), with females and those in the novel context consuming less. All groups increased their consumption across the 5 habituation and testing sessions, and the rate at which consumption increased significantly differed across groups ($F(1,28)=227.398$, $p<0.01$), with rats tested in the novel context habituating slower to the novel food ($F(1,28)=11.396$, $p=0.002$). Across habituation sessions, rats in the novel context consumed significantly less than those tested in their home cage ($F(1,28)=11.451$, $p=0.002$), while sex approached significance during the final test ($F(1,28)=3.259$, $p=0.082$), with females consuming less. The Fos induction analysis within the BMAa found that the females tested in the novel context had the highest number of Fos positive neurons. Preliminary analyses of the BMAa (n=3-4/group) confirmed that females had a significantly higher number of Fos positive neurons than males ($F(1,9)=8.837$, $p=0.026$), and, while not significant, rats tested in the novel context had more Fos induction than those tested in their home cage ($F(1.9)=4.312$, $p=0.068$). Collectively, these results suggest that the BMAa is a part of the network that controls consumption during habituation to a novel food in a novel environment, and that increased activity in the BMAa may be correlated with suppression of feeding and sex differences.

Disclosures: Z. Irving: None. **E. Greiner:** None. **G. Petrovich:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDDK grant DK085721

Title: Activation of the amygdala & paraventricular thalamus after novelty exposure during consumption in male and female rats

Authors: *E. GREINER[1], G. D. PETROVICH[2];
[2]Psychology, [1]Boston Col., Chestnut Hill, MA

Abstract: Adaptive reactions to novel stimuli are essential for survival. One of the most important interactions with novel stimuli is with novel foods. Previously we found that novel food and novel context inhibited food intake in hungry male and female rats and induced Fos in the central amygdala (CEA). We also determined that in a novel context, males habituated to novel food faster than females, who maintained suppressed consumption through multiple exposures. Still, very little is known about the neural substrates that mediate the impact of novelty on consumption and underlie the behavioral sex differences. To fill this gap in knowledge, we analyzed Fos induction in key brain areas connected to the CEA in male and female rats that were exposed to either a novel or familiar food in a novel or familiar environment. Following 20h acute food deprivation, male and female Long Evans rats (n=8/group) were tested for consumption in either their home cage or in a novel context and were given either a familiar (rat chow) or novel (Test Diet pellets; TD) food. Rats were perfused 90 minutes after the start of testing and their brains were immunohistochemically processed for c-fos expression.Our analysis focused on the cell groups in the basolateral complex (basolateral, basomedial (BMA), and lateral nuclei of the amygdala) and the paraventricular nucleus of the thalamus (PVT). Within the basolateral complex, there was greater Fos induction in all cell groups for rats tested in a novel context with additional greater Fos induction within the anterior BMA for rats given a novel food. Analysis of the anterior PVT revealed greater Fos induction for rats tested in a novel context and for rats given a novel food. The effect of novel food was driven entirely by females who had greater Fos induction to a novel food than a familiar food, regardless of context, while males did not. Preliminary analysis of the posterior PVT indicates a similar pattern of activation to the anterior PVT. These results indicate that the basolateral complex and PVT are parts of the network that controls consumption when rats are exposed to novel foods or are fed in novel environments, providing important contextual information and

sex-specific activation to downstream targets, like the CEA. This work is the first step to establish the neural circuitry that mediates the impact of novelty on consumption.

Disclosures: E. Greiner: None. **G.D. Petrovich:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DK085721

Title: Behavioral examination of sex differences in food motivation and consumption under physiological satiety and hunger

Authors: *R. SHTEYN, D. S. LAFFERTY, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Hunger and palatability could impact food consumption in concert or independently through homeostatic and hedonic mechanisms. A recent study from our group found sex differences in consumption of high-sugar pellets (Test Diet; TD) that suggested that females are particularly prone to consume palatable food regardless of hunger state. Males consumed TD consistent with their hunger state—fasted consumed more than sated, while sated females ate as much as fasted females. These sex differences were not observed in the consumption of standard rat chow, suggesting that food motivation for palatable food may be differently impacted by hunger and satiety in males and females. In addition to consumption, food motivation influences food-seeking behaviors and learning about food cues. Here we compared how both sexes learn about and consume palatable food under sated and hungry conditions during Pavlovian conditioning, extinction, and context-mediated renewal of responding. Male and female adult Sprague Dawley rats were either food restricted to 85% of their *ad libitum* body weight (BW) or fed chow *ad libitum* throughout the experiment (n=8 per group, 4 groups). Rats learned cue-food associations across 8 training sessions, each with eight tone (conditioned stimulus; CS) presentations immediately followed by two TD pellets (unconditioned stimulus; US). Following 4 days of extinction (CS-only presentations), rats were tested for renewal of conditioned responding (defined as time spent in the food cup during CS presentation) over two days, with one session taking place in the acquisition context and one in the extinction context, counterbalanced. Prior to and following all learning and testing sessions, rats were tested for consumption of TD and chow. Each test was conducted over two days with one-hour access to TD or chow in the home cage, with food type order counterbalanced. Preliminary consumption test analyses (grams eaten expressed as percent of BW) found that hungry rats ate significantly

more TD and chow than sated rats regardless of sex ($p<.001$). There were no significant differences in TD consumption between chronically hungry females and males during either the test that occurred before ($p=.082$) or after ($p=.121$) training. Importantly, sated females ate more TD than sated males during each test ($p=.003$, $p=.006$). These results suggest that females are more sensitive to palatable food when sated, but when chronically hungry, the drive to consume palatable and neutral-tasting food is similar for both sexes. Pending analyses will assess conditioned responding during cue-food acquisition, extinction, and renewal, and if they correlate with consumption in each sex.

Disclosures: R. Shteyn: None. **D.S. Lafferty:** None. **G.D. Petrovich:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01DA035943

Title: Dopamine correlates of habit versus goal-directed behavior in ventral tegmental area

Authors: *R. MAGNARD[1], P. H. JANAK[1,2], Y. VANDAELE[1];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Habitual performance is proposed to be mediated by a shift in reward seeking control from mesolimbic to nigrostriatal dopaminergic pathways. However, how midbrain dopamine (DA) neuron activity differentially signals habitual versus goal-directed performance remains largely unknown. To address this question, we tested male and female WT rats in two instrumental procedures, a lever insertion fixed-ratio 5 (LI5) task (n=7 males; 8 females) and a lever retraction fixed-ratio 5 (LR5) task (n=7 males; 8 females). In these tasks, the timing of lever insertion and retraction is varied so that insertion or retraction is a relevant cue signaling reward (sucrose 20%) availability while the other cue is made irrelevant. Rats were trained for 30 or 45 min daily sessions in the LR5 and LI5 respectively, to allow similar lever availability time. We then tested the goal directed or habitual behavior in satiety induced devaluation test. Finally, we compared activity of DA neurons via *in vivo* fiber photometry after VTA infusion of Cre-dependent GCaMP6f virus in adult TH-Cre rats, performing either LR5 (n=4 males; 2 females) or LI5 (n=1 male; 4 females) task, while DA neuron activity was monitored. We found for both male and female rats, behavior quickly becomes automatic and habitual in the LR5 task in which the LR cue predicts immediate reward delivery, as indicated by tests of satiety induced reward devaluation. We further observed a rapid shift in the activation of DA VTA neurons from reward

retrieval to the earlier LR cue, followed by a decrease in cue-related DA signals across repeated trials as rats develop habitual behavior. In contrast, behavior in the LI5 task, was goal-directed, and cue-induced DA activation remained relatively constant across trials and sessions, when reward availability was signaled by the LI cue, more distal from reward delivery. These results show manifest task differences in DA signaling that appear consistent with the difference in habitual versus goal-directed control of behavior.

Disclosures: R. Magnard: None. **P.H. Janak:** None. **Y. Vandaele:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH U19NS113201
NIH R35NS105107
NIH F32DA035543
NIH U01NS013522
NIH U01NS090604
HHMI
Sackler Scholar Programme in Psychology

Title: Asynchronous modulation of striatal PKA activity by dopamine during learning

Authors: *S. LEE[1], B. LODDER[1], Y. CHEN[1], T. PATRIARCHI[2], L. TIAN[2], B. SABATINI[1]; [1]Howard Hughes Med. Inst. - Harvard Med., Boston, MA; [2]Biochem. and Mol. Med., Univ. of California, Davis, Davis, CA

Abstract: Reinforcement learning models involving dopamine and basal ganglia postulate that midbrain dopamine neurons encode information about action and action outcome and provide a teaching signal to striatal spiny projection neurons in the form of dopamine release. Dopamine, in turn, is thought to guide learning via differential modulation of downstream protein kinase A (PKA) in each class of spiny projection neurons (SPNs). However, the real-time relationship between dopamine and SPN PKA activity during learning has not been directly tested due to the difficulty of measuring intracellular kinase activity in behaving animals. To address this limitation, we have developed a fluorescence lifetime photometry system to measure SPN PKA activity in freely behaving animals. Combining this technique with behavior, pharmacology, fiber photometry, and optogenetics, we have successfully measured the level of dopamine and the net PKA activity in each class of SPNs in the nucleus accumbens and found that positive and negative modulation of dopamine that evolves across training is both necessary and sufficient to

explain concurrent fluctuation in the PKA activity. More specifically, modulations of PKA in SPNs that express type-1 and type-2 dopamine receptors were dichotomous and asynchronous such that PKA activity was sensitive to increase and decrease in dopamine, respectively, that occurs at different phases of learning. Furthermore, employing cell-type specific viral overexpression of peptide inhibitor of PKA, we show that PKA activity in each class of SPNs contributes to reward based learning.

Disclosures: S. Lee: None. **B. Lodder:** None. **Y. Chen:** None. **T. Patriarchi:** None. **L. Tian:** None. **B. Sabatini:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH grant R01-NS101356
CNPq Science without Borders Scholarship 203059/2014-0
Larry Deaven Ph.D. Fellowship in Biomedical Sciences

Title: An operant memory is encoded as a shift in neuronal population dynamics

Authors: *R. M. COSTA[1], D. A. BAXTER[2], J. H. BYRNE[1];
[1]Dept. of Neurobio. and Anat., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX;
[2]Engin. and Med., Texas A&M Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Learning engages multiple brain regions and multiple neurons within each region, creating a high-dimensional neuronal population space. Yet, it remains unclear how learning reconfigures this high-dimensional space and whether it is possible to identify a lower-dimensional "*learning signature*". Here, we investigated this issue using an *in vitro* analogue of operant conditioning of *Aplysia* feeding behavior and single-neuron resolution voltage-sensitive dye imaging. Contingent training consisted of stimulating a dopamine-rich nerve following occurrences of ingestion buccal motor patterns (iBMPs) in isolated buccal ganglia. Training in the contingent group resulted in a significant increase in the rate of iBMPs relative to the yoked control group (N = 7, Wilcoxon signed-rank, P = 0.03), which received identical stimulation irrespective of iBMP occurrence. We recorded the activity of ~100 neurons per preparation during BMPs before and after contingent and yoked training. Although no significant difference was observed between groups in the overall firing rate (P = 0.81), the overall correlation among pairs of neurons was higher in the contingent group compared to the yoked group (P = 0.03). This result suggested that contingent training modified the relationship among the activities of multiple cells. To gain additional insights, we used a dimensionality reduction approach (non-

negative matrix factorization) to identify ensembles of coactive neurons. Two ensembles were identified that were highly consistent across preparations: a protraction module, which underlies the phase of BMPs responsible for forward movement of the animal's feeding apparatus; and a retraction module, which underlies the phase responsible for the backward movement. Next, we examined how recruitment of these modules over time was modified by training. We found that recruitment of the retraction module ($P = 0.02$), but not the protraction module ($P = 0.94$), was significantly advanced in time in the contingent group relative to the yoked group. We did not observe a difference between groups in the level of recruitment of either module (retraction, $P = 0.30$; protraction $P = 0.81$). In addition, each module displayed characteristic spatial distribution of neuronal somata across preparations, which was consistent with previously described protraction and retraction neurons. These results suggest that the primary signature of operant learning can be identified as a shift in recruitment of a lower-dimensional module, and points to the location of neurons contributing to that module.

Disclosures: R.M. Costa: None. **D.A. Baxter:** None. **J.H. Byrne:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R00 DA042895
NIH Grant T32 DA007234

Title: The role of superior colliculus inputs to midbrain dopamine centers in appetitive Pavlovian conditioning

Authors: *C. L. POISSON[1], C. R. HERUBIN[1], B. T. SAUNDERS[2];
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Abstract: Appetitive Pavlovian conditioning relies on associations made between sensory stimuli ("cues") and rewarding outcomes. In our previous studies, we have shown that optogenetic activation of dopamine (DA) neurons in the ventral midbrain in the presence of a neutral visual cue is sufficient to create a Pavlovian association that promotes conditioned behavior in response to that cue (Saunders et al., 2018). Critically, the circuit mechanisms underlying the integration of cue-related sensory input into DA centers to form Pavlovian associations remain unclear. One candidate structure that could provide visual information about cues is the superior colliculus (SC), which receives direct retinal input, and sends projections to DA neurons in the ventral tegmental area (VTA) and substantia nigra pars compacts (SNc). The SC is known to be necessary for cue-evoked neural activity in DA neurons, but the behavioral

function of SC-VTA/SNc neurons is unclear. Here, we characterize the SC-VTA/SNc circuit and use optogenetics to modulate this pathway's activity during Pavlovian conditioning in rats. First, we used a dual-virus approach to determine which SC neurons innervate the ventral midbrain. This revealed projections from deep-layer SC that largely innervate the dorsal and lateral VTA and SNc. To manipulate activity of the pathway, we injected viruses expressing excitatory (ChR2) or inhibitory (eNpHR) opsins into deep layer SC and implanted an optic fiber in either VTA or SNc. We next trained rats in a Pavlovian conditioning procedure wherein visual cues were paired with optogenetic manipulation of terminals. Behavioral analysis revealed that excitation of these neurons failed to drive conditioned (i.e., learned) behavior towards the cue, in contrast to our previous studies targeting DA neurons directly. Notably, a subset of ChR2 rats demonstrated turning behavior. Turning was contralateral to stimulation hemisphere, and time-locked to laser duration. This suggests that the SC-VTA/SNc neurons can drive movement invigoration, presumably via their activation of DA neurons, but do not directly cause Pavlovian learning. In ongoing experiments, we are testing the working hypothesis that subsets of SC inputs to different DA populations may invigorate actions, and/or alter attention to relevant sensory information during learning.

Disclosures: C.L. Poisson: None. **C.R. Herubin:** None. **B.T. Saunders:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant 5T32DK007247-43
NIH Grant F32DA053714-01
Wellcome Trust 204915/Z/16/Z
Whitehall Foundation 2019-08-72

Title: Brain-wide neural encoding of value and salience across need states

Authors: *A. J. BOWEN, D. J. OTTENHEIMER, N. A. STEINMETZ;
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Abstract: Value and salience are interrelated components of cognition that are thought to arise from distinct systems. As such, they are frequently studied independently in distinct brain regions. Here, we sought to evaluate the extent to which regions classically studied in relation to these cognitive variables exhibit specialized encoding. We designed a behavioral task that features integration of these components, in which thirsty mice were presented with stochastically rewarded (CS+) and unrewarded (CS-) Pavlovian cues. The probability that

sucrose delivery followed the CS+ varied across the session. On some trials, reward was replaced with highly salient white noise. Mice adapted their anticipatory licking for the CS+ to the reward history: when recent trials were frequently rewarded, mice licked more robustly. They also demonstrated distinct licking patterns to reward, reward omission, and white noise, and licking decreased dramatically across the session as mice became sated. Thus, this task permits the disambiguation of value and salience. Once mice were trained, we recorded from many thousands of neurons in a variety of cortical and subcortical regions using Neuropixels 2.0 probes. Targeted regions included ones associated with value, salience, and/or satiety (OFC, mPFC, NAc, LHA, NTS, PBN, CeA). The activity of a majority of neurons in all recorded regions was modulated following reward delivery. Preliminary analysis revealed neurons with a variety of coding properties. Some were tuned to satiety-gated salience, with decreasing activity following rewards but maintained white noise-driven activity across the session. Others were especially tuned to value, with reward responses modulated by reward history. These results begin to distinguish the unique coding properties of a variety of regions often associated with value, salience, and satiety.

Disclosures: A.J. Bowen: None. **D.J. Ottenheimer:** None. **N.A. Steinmetz:** None.

Poster

P666. Neural Systems III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P666.10

Topic: G.02. Reward and Appetitive Learning and Memory

Title: C-fos activation mapping of hierarchical vs. binary associative structures in reward prediction

Authors: *S. PETERSON, A. MAHERAS, J. I. CHAVIRA, B. WU, R. KEIFLIN;
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Abstract: Research into the neural basis of reward prediction has often focused on predictions resulting from a binary association between some environmental stimuli and an ensuing rewarding outcome. Importantly, reward predictions and reward-seeking behaviors are also controlled by hierarchical associative processes. This refers to the acquired property of certain stimuli (often contextual stimuli) to inform subjects about the validity of ambiguous reward-paired cues (e.g., animals can learn that a reward cue is valid —i.e. results in reward— only if encountered in a certain context). Empirical behavioral studies and neural network simulations indicate that hierarchical and simple binary associations rely on different associative structures, suggesting partially dissociable neural substrates for these forms of reward predictions. Here we aimed to generate and compare the brain-wide activation maps corresponding to these two forms of reward prediction (binary vs. hierarchical associations). Three groups of rats were trained in

three different forms of Pavlovian discriminations. A first group of rats was trained in a context-dependent Pavlovian discrimination task, in which the validity of two brief auditory tones (A and B) to signal reward depended on contextual visual background (Cx1 and Cx2, alternating every 2 mins). Specifically, cue A was rewarded only in the Cx1 but not in the Cx2, while the opposite was true for cue B (Cx1: A^+ / B^- ; Cx2: A^- / B^+). A second group was trained in a simple Pavlovian discrimination task in which one cue reliably signaled reward in both contexts while the other cue was never paired with reward (Cx1: A^+ / B^- ; Cx2: A^+ / B^-). Finally, for a third group both auditory cues were ambiguous predictors of reward, regardless of the context (Cx1: $A^{+/-}$ / $B^{+/-}$; Cx2: $A^{+/-}$ / $B^{+/-}$). Therefore, all rats were exposed to the same cues, contexts, and rewards but relied on different associative structures to predict and seek reward. Following acquisition and maintenance of stable performance in these tasks, we collected the brains of all rats 120 min after the start of their last behavioral session and performed immunofluorescence mapping of c-fos expressing neurons in a selected set of structures. We observed that performance in these different tasks resulted in partially dissociable neural activation maps, presumably reflecting the different associative or cognitive processes engaged in these tasks. Of critical importance was the claustrum, which showed a strongly reduced activation in context-depended discrimination. This suggests that the claustrum could constitute a critical hub in the hierarchical control of reward seeking.

Disclosures: S. Peterson: None. **A. Maheras:** None. **J.I. Chavira:** None. **B. Wu:** None. **R. Keiflin:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.01

Topic: G.03. Motivation

Support: P50 MH119467-02
P30 DA048736

Title: The ventral tegmental area nociceptin system is engaged during stress and regulates motivated behaviors

Authors: *C. STINE, D. J. MARCUS, J. HANSEN, M. R. BRUCHAS;
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Abstract: Major depressive disorder (MDD) is a significant global health concern affecting over 160 million people worldwide. A central feature of MDD is anhedonia, which presents as abnormal approach-avoidance and loss of motivation. It is critical to understand the neural circuitry regulating expression of anhedonia in order to identify new, more effective therapeutic

targets. Our laboratory previously showed that nociceptin neurons in the paranigral ventral tegmental area (pnVTA^{Pnoc} neurons) act locally on nociceptin receptor (NOPR) expressed on dopamine neurons to limit motivation for reward, and upon activation can induce aversion (Parker et al., 2019, Cell). Because nociceptin has been implicated in the regulation of stress-related behavior, we hypothesize that stress engages the pnVTA nociceptin/NOPR system to regulate the expression of motivational deficits. To test this, we injected a Cre dependent genetically encoded calcium indicator (GCaMP6s) into the pnVTA of Pnoc-Cre mice and recorded prepronociceptin neuron activity during exposure to a range of stressful stimuli including open field test, elevated zero maze, aversive odor, tail lift, simulation of a looming predator, and foot shock. Fiber photometry recordings showed that exposure to each of these stressors induces nociceptin neuron activation, supporting our hypothesis that stress engages the pnVTA nociceptin system. To further test this nociceptin population's effect on motivation, we injected a Cre virus into the pnVTA of our Pnoc conditional knockout mice to induce loss of nociceptin. We then placed these mice through the progressive ratio test to determine how well they perform a task in order to receive a reward which exponentially increases in required effort with each trial, ultimately reaching a "breakpoint". The progressive ratio test showed that mice without pnVTA nociceptin were more motivated for reward than their control counterparts, obtaining more rewards and reaching a higher motivational breakpoint. Lastly, we completed retrograde tracing to identify GABAergic and glutamatergic inputs onto pnVTA neurons which labeled multiple afferent regions including the lateral hypothalamus (LH). Further electrophysiology studies confirmed that the LH has both GABA and glutamatergic connectivity onto pnVTA nociceptin neurons. Ultimately, these findings provide evidence that pnVTA nociceptin release constrains motivation for reward, identify potential afferent inputs that regulate pnVTA nociceptin activity, and offer new insight into stress-related behavioral states that engage this population. Supported by: P50 MH119467-02, P30 DA048736.

Disclosures: C. Stine: None. **D.J. Marcus:** None. **J. Hansen:** None. **M.R. Bruchas:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.02

Topic: G.03. Motivation

Support: NIH Grant R37DA033396
Mary Gates Research Scholarship
Scan Design Innovations in Pain Research

Title: Dynorphinergic control of amygdalo-striatal circuits for goal-directed action

Authors: *R. GOWRISHANKAR[1], A. J. ELERDING[1], S. E. SHIRLEY[1], A. A. GORDON-FENNELL[1], C. ZHOU[1], L. TIAN[2], G. D. STUBER[1], M. R. BRUCHAS[1];
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Abstract: Elegant work spanning decades has implicated the dorsomedial striatum (DMS) in how animals learn, perform and update goal-directed actions. Yet, the circuit- and molecular-level substrates involved in these behaviors remain largely unknown. Given the amygdala's role in encoding value, neural projections from specifically the basolateral amygdala (BLA) to the DMS are uniquely poised to sculpt action-outcome behaviors. Roughly 50% of the neurons in the DMS express dynorphin; although how dynorphin (dyn) itself coordinates these strategies is yet to be elucidated. Interestingly, ours and prior studies have shown that BLA neurons project exclusively to dynorphin neurons in the DMS and that DMS-projecting BLA neurons express kappa-opioid receptors (KOR). ***Hence, we hypothesize that BLA projections to the DMS coordinate outcome-based action learning and maintenance, under the control of dyn-KOR signaling.*** Here, we show that retrograde dyn transmission at BLA terminals in the DMS inhibits BLA-DMS activity during outcome delivery, refines increased activity during action, and promotes action-outcome behaviors for natural reward. Using fiber photometry in BLA terminals expressing GCaMP6s in the DMS, we show that BLA terminals are active during action and inhibited during outcome. We also observe that BLA-DMS activity changes dynamically and reversibly to manipulations in outcome-probability or outcome-value. Notably, we find that this engagement is sensitive to disruptions in dyn signaling via naloxone or through local deletion of dyn in the DMS, or KOR in the BLA. Using a novel dyn sensor (KLight1.3), we observe that DMS dyn is released during reward retrieval, suggesting that dyn release and subsequent KOR signaling inhibits BLA-DMS activity during outcome, following pellet retrieval. In parallel, using channelrhodopsin (ChR2) or a light-activatable Gi/o-coupled GPCR, parapinopsin (PPO) for time-locked optogenetic manipulations of BLA-DMS terminals, we find that photoactivation during outcome disrupts action and conversely, photoinhibition using enhances actions, suggesting that BLA-DMS activity during action is informed by outcome. ***Collectively, our data suggest that BLA-DMS activity informs outcome-dependent action and is regulated by dyn-KOR signaling at these terminals during outcome.*** Current work is focused on delineating how BLA-DMS axons differentially engender action or outcome using *in-vivo* 2-photon calcium imaging of DMS-projecting BLA neurons or BLA axon clusters in the DMS, and how their activity is differentially controlled by local dynorphin signaling in the DMS, during a novel, head-fixed operant task.

Disclosures: R. Gowrishankar: None. **A.J. Elerding:** None. **S.E. Shirley:** None. **A.A. Gordon-Fennell:** None. **C. Zhou:** None. **L. Tian:** None. **G.D. Stuber:** None. **M.R. Bruchas:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.03

Topic: G.03. Motivation

Support: NIDA R00 045765

Title: The dual orexin receptor antagonist suvorexant reduces cocaine behaviors at non-sedating doses

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Abstract: Introduction: Blocking orexin (hypocretin) signaling is a promising novel strategy for the treatment of cocaine use disorder. Suvorexant is a dual orexin receptor antagonist that is FDA-approved for the treatment for insomnia and thus could be readily repurposed. Although there is some evidence in rat to indicate that suvorexant can reduce cocaine-related behaviors, this has not been tested in a model of cocaine self-administration that promotes an addiction-like phenotype. Moreover, the dose-response profile of suvorexant when administered via a clinically relevant route (p.o.) has not been examined, nor is it known if there are sedative effects at doses required to reduce cocaine behaviors. Thus, here we tested the efficacy of suvorexant (delivered p.o.) on the expression of two hallmark addition phenotypes and general activity in rats exhibiting a paradigmatic-induced addiction-like state. **Methods:** Male Long Evans rats (n=16) were assessed for baseline economic demand for cocaine using a within-session threshold procedure. Rats were then given daily intermittent access to cocaine (5min access every 30min for 6h) for 2 weeks before being reassessed for economic demand and compulsive (punished) responding for cocaine. The effect of suvorexant (0, 3, 10, 30mg/kg; oral gavage) on demand and compulsive responding was assessed. Potential sedative effects of suvorexant were assessed using a locomotor reactivity task. **Results:** Intermittent access to cocaine was associated with a significant decrease in demand elasticity for cocaine (increased motivation) which was reversed by suvorexant (10, 30mg/kg). Suvorexant was also effective at reducing compulsive (punished) responding for cocaine. At the minimum effective dose of suvorexant (10mg/kg), no sedative effects were observed on the locomotor activity test. **Discussion:** In rats that transitioned to an addiction-like state, suvorexant was effective at reducing key addiction endophenotypes at doses that are non-sedating, supporting the potential repurposing of this compound for the management of cocaine use disorder. Ongoing studies are testing for any effects of the minimum effective dose on cognitive/psychomotor outcomes, as well as the efficacy of suvorexant in reducing addiction behaviors in females.

Disclosures: S.L. O'Connor: None. **M.M. Bilotti:** None. **M.H. James:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.04

Topic: G.03. Motivation

Support: R01 MH121603
F31 MH125659

Title: Connectional Architecture of Sex-Different Vasopressin Neurons in the Bed Nucleus of the Stria Terminalis

Authors: *N. RIGNEY[1], G. J. DE VRIES[2], A. PETRULIS[2];
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Abstract: The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication. Ablations of the sex-different AVP-expressing cells within the bed nucleus of the stria terminalis (BNST) result in sex-different effects on these behaviors. Despite the substantial body of work implicating AVP in social behavior, evidence for inputs and outputs of BNST AVP cells is largely circumstantial. Here, we use a Cre-dependent, modified rabies virus (RV; for retrograde tracing) and a synaptophysin-tagged virus (for anterograde tracing) in combination with AVP-iCre (or iCre negative control) mice to map out monosynaptic inputs and outputs of BNST AVP cells. Adult male and female mice received unilateral injections of adeno-associated viruses (AAVs) expressing either Cre-dependent, TVA-eGFP (for driving cell infection by RV) and glycoprotein (for transsynaptic retrograde infection), or GFP-2A-Synaptophysin-mRuby into the BNST. For RV tracing, mice also received an injection of RV following a 10-day incubation period. Preliminary results indicate that BNST AVP cells in male mice show strong reciprocal connections with the lateral septum, ventral pallidum, medial amygdala, lateral and medial preoptic area, and premammillary nucleus, all regions known to be innervated by male-biased, sex-different AVP fibers. Whereas BNST AVP cells receive inputs from nucleus accumbens and the paraventricular nucleus of the hypothalamus, they do not project to these regions. In addition, BNST AVP cells also receive strong inputs from, but project moderately to, the lateral, anterior, and ventromedial hypothalamic areas. Conversely, BNST AVP cells receive few inputs from, but strongly project to, the lateral habenula, periaqueductal gray, dorsal raphe, piriform cortex, and anterior olfactory nucleus. Lastly, BNST AVP cells send moderate outputs to, but do not receive inputs from, the horizontal diagonal band and entorhinal cortex. Collectively, we establish that brain regions previously shown to contain sex-different AVP innervation receive projections from BNST AVP cells, while these areas (i.e., lateral septum, ventral pallidum) also provide input to drive these cells. The BNST and medial amygdala also display profound reciprocal connections, and therefore may function as a system for the regulation of sexually different social behavior. This information will be foundational for understanding sexually different function of AVP circuitry and future studies will differentiate inputs/outputs of AVP cells in the BNST and medial amygdala in both sexes.

Disclosures: N. Rigney: None. **G.J. De Vries:** None. **A. Petrulis:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.05

Topic: G.03. Motivation

Support: NIH grant MH-114026-02

Title: Sex differences in the neural circuits underlying voluntary exercise behavior

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Abstract: Despite the beneficial effects of exercise on physical and psychological health, most people fail to achieve recommended levels of physical activity. Identifying the circuits that drive exercise behavior could lead to novel strategies to increase exercise participation and maximize the benefits of exercise, but the neural mechanisms governing voluntary exercise are unknown. Rats maintain high levels of voluntary wheel running (VWR) that consists of two distinct phases: acquisition and maintenance. During the acquisition phase, rats escalate their nightly running before reaching a plateau in the maintenance phase. This pattern of VWR closely resembles that of other appetitive operant behaviors, such as lever pressing for sucrose, suggesting that similar mechanisms may underlie both behaviors. Dopamine (DA) in the dorsomedial striatum (DMS) is known to support the initial acquisition of operant behavior, while DA in the dorsolateral striatum (DLS) governs its long-term maintenance. Interestingly, the acquisition phase of VWR is shorter in female rats than it is in males, suggesting that DLS-control over voluntary exercise might be facilitated in females compared to males. The goal of these experiments was to identify the circuits governing the acquisition and maintenance of VWR and determine if sex differences exist. We inactivated the DMS or DLS of adult, Long-Evans rats by injecting a GABAA/GABAB agonist into the DMS or DLS before a VWR bout during both the acquisition phase (4 d) and maintenance phase (4 wk) of wheel running. In males, inactivation of the DMS reduced VWR during the acquisition phase, but not the maintenance phase. In contrast, inactivation of the DLS reduced VWR during the maintenance phase but had no effect during the acquisition phase in males. In females, inactivation of the DMS had no effect on VWR in either phase, but DLS inactivation reduced VWR during both phases. Moreover, double fluorescent in situ hybridization revealed that VWR recruits D1-expressing neurons in the DLS more rapidly in females (days) than it does in males (weeks). These data suggest that the neural circuits that support voluntary exercise behavior depend on both the phase of exercise and sex. Results

illustrate there are sex differences in the recruitment of the DLS, which have the potential to reveal mechanisms implicated in exercise motivation and maintaining exercise behavior.

Disclosures: N. Jamil: None. **M.K. Tanner:** None. **K. Abrams:** None. **A. Hohorst:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.06

Topic: G.03. Motivation

Support: NIDDK Grant R01DK106229

Title: Trans-synaptic Investigation of Upstream Regulators of the Mesolimbic Pathway

Authors: *K. OGUNSEYE[1], H. SUN[2], J. KASPER[2], J. HOMMEL[2];
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Abstract: The mesolimbic pathway is well-characterized as an important mediator of motivated behavior for substances of abuse, highly palatable food, and other reinforcers. However, it remains unclear which specific neurons project onto the mesolimbic pathway. Typically, mono-synaptic (i.e., region A → region B) viral tracing techniques have been utilized to investigate mesolimbic neurocircuitry. Utilizing novel trans-synaptic viral techniques, in which at least three brains regions can be mapped concurrently (i.e., region A → region B → region C), we aim to trace upstream brain regions acting as regulators of the ventral tegmental area (VTA) → nucleus accumbens (NAc) mesolimbic pathway. We used stereotaxic surgical methods to microinject a retrograde adeno-associated virus (AAV) 6 expressing the avian derived receptor TVA (CMV AAV6 TVA mcherry) into the nucleus accumbens of 11-19 week-old C57BL/6 male mice. After adequate expression of AAV6, we injected a G-protein deleted rabies virus enveloped by the avian sarcoma leukosis virus receptor (EnvA G deleted GFP Rabies) into the VTA. Utilizing immunohistochemical studies, we observed appropriate mcherry AAV6 expression at the primary site of infection, the NAc, and expression of GFP rabies in the VTA. Rabies expression was also identified in the lateral hypothalamus, periventricular region of the hypothalamus, sub paraventricular region of the hypothalamus, throughout the nucleus accumbens, and prelimbic and infralimbic cortex indicating that these brain regions project directly onto the VTA → NAc pathway. These results demonstrate successful neuro-tracing of inputs into the mesolimbic pathway. This study highlights neuroanatomical evidence of upstream regions that may have modulatory effects specific for directing mesolimbic activity and function. This viral-tracing

strategy supports the elucidation of inputs more broadly into neural pathways instead of individual brain regions.

Disclosures: K. Ogunseye: None. **H. Sun:** None. **J. Kasper:** None. **J. Hommel:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.07

Topic: G.03. Motivation

Support: National Science Foundation Graduate Research Fellowship Program, DGE 1256260 (AI)
R25-NS080686-11/12 NIH-NINDS (BR)
R01 DA 038599 (SBF)
T32DA07268 (SW)

Title: The effects of selective inhibition of orexinergic projections from the lateral hypothalamus to the paraventricular nucleus of the thalamus on cue- and food-motivated behavior

Authors: *A. G. IGLESIAS[1], B. DE LA REA[2], S. WESTBROOK[1], S. B. FLAGEL[1]; [1]Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The survival of an organism is dependent on their ability to properly respond to cues in the environment. In rodents, individual differences in cue-motivated behaviors appear in a Pavlovian conditioned approach (PavCa) paradigm, wherein a discrete cue is followed by delivery of a food reward. Some rats, sign-trackers (STs), attribute excessive incentive motivational value to reward cues, which thereby transforms the cues into attractive and desirable stimuli that can gain inordinate control and lead to maladaptive behavior. Other rats, goal-trackers (GTs), primarily attribute predictive value to reward cues. Prior work using the ST/GT model has implicated the paraventricular nucleus of the thalamus (PVT) as a neural hub responsible for mediating differences in Pavlovian incentive learning, and orexinergic projections from the lateral hypothalamus (LH) to the PVT seem to be important in this regard. Lesions of the LH decrease the propensity to attribute incentive motivational value to reward cues, and administration of orexin (OX) antagonists into the PVT reduces the incentive value of reward cues. Thus, we postulate that orexinergic projections from the LH relay the incentive value of reward cues to the PVT. To address this hypothesis, we utilized transgenic OX-Cre Long Evans rats, which express Cre-recombinase in OX neurons. We selectively inhibited OX neurons projecting from the LH to the anterior PVT (aPVT) by infusing a retrograde Cre-dependent inhibitory (Gi) DREADD (Designer Receptors Exclusively Activated by Designer

Drugs) into the aPVT and administering systemic (i.p.) clozapine-N-oxide (CNO, 5 mg/kg). Adult male and female OX-Cre rats were characterized as STs or GTs following 5 PavCA sessions. To determine if inhibition of OX neurons in the LH-PVT pathway affects the incentive value of a reward cue, CNO or vehicle (VEH) was administered prior to a conditioned reinforcement task, where rats had to elicit an instrumental response for the lever-cue previously paired with reward. CNO-treated rats showed less responding for the lever-cue. To determine whether inhibition of OX neurons in the LH-PVT pathway had a general effect on motivated behavior and instrumental responding, CNO or VEH was administered prior to a fixed-ratio (FR) 1 task for food reward. Gi-DREADD activation did not appear to affect FR1 responding for small or large amounts of food reward. Thus, inhibition of OX neurons in the LH-PVT pathway diminishes the incentive motivational value of reward-cues, without affecting the valence of the reward itself. This orexinergic LH-aPVT pathway may therefore play a critical role in encoding the incentive value of reward-cues.

Disclosures: A.G. Iglesias: None. **B. de la Rea:** None. **S. Westbrook:** None. **S.B. Flagel:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.08

Topic: G.03. Motivation

Title: High resolution MRI guided neuroanatomical tracing in Rhesus macaque

Authors: *A. ALIKAYA, D. SCHAEFFER, A. C. SILVA, W. R. STAUFFER;
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Abstract: Dopamine projections to the striatum and cerebral cortex are heavily involved in learning, reward processing, movement, and decision making. However, the relative organization of striatal- and cortical-projecting dopamine neurons in the midbrain remains to be resolved. Neuroanatomical tracing can provide a wealth of information about the organization of neural circuits, but is highly dependent on the accuracy of tracer injection sites. Achieving millimeter-level precision and replicability of injection sites is particularly challenging when targeting small subcortical structures in large brains. High-resolution MRI scans are imperative in making such precise tracer injections. Here, we developed an approach using gadolinium (Gd) guided high-resolution MRI to inject retro-AAV into functionally related regions of the caudate nucleus and frontal cortex, and optimized scanning sequences to improve the visualization of the injection needle. We designed and built an MRI-compatible stereotaxic frame that enabled us to image Rhesus macaques in the supine position, using standard, clinical human MRI coils. We first used a high-field clinical (7T) MRI scanner to provide high-resolution images of baseline scans. We

used subsequent scans to monitor the stepwise advancement of a Gd filled injection needle into the brain through a chronically implanted chamber. The injection needle consisted of two pieces of silica tubing sealed together with cyanoacrylate to create a reflux-resistant cannula. We optimized MP2RAGE and susceptibility-weighted imaging (SWI) sequences to observe the advancement of the injection needle into intermediate regions of the associative caudate and to best resolve the coordinates of the Gd doped cannula at the injection site. Once we confirmed the location of the needle in the intermediate caudate, we used convection enhanced delivery to inject 10µl of virus doped with Gd (250:1), and used MP2RAGE MRI pulse sequences to verify the location. The contrast agent allowed for clear visualization of the spread of virus in real time. The methods described here will be applicable for precise targeting of other deep brain structures, and overall help improve accuracy and replicability in neuroanatomical tracing of circuits in large nonhuman primate brains.

Disclosures: A. Alikaya: None. **D. Schaeffer:** None. **A.C. Silva:** None. **W.R. Stauffer:** None.

Poster

P675. Motivation: Subcortical Neurocircuitry II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P675.09

Topic: G.03. Motivation

Support: NIH Grant DA037327
NIH Grant DA054370

Title: More than a gut feeling: cholecystokinin afferents to the nucleus accumbens encode aversion and modulate dopamine transmission

Authors: O. P. CULVER[1], J. PARRILLA-CARRERO[2], *T. JHOU[2];
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Abstract: Originally recognized for its role in regulating gastrointestinal physiology, cholecystokinin (CCK) is also one of the most abundant neuro-modulatory peptides in the brain, and is highly expressed in brain regions that regulate emotional processing and motivational behavior. These regions include the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral tegmental area (VTA). Using CCK-Cre mice crossed to reporter mice, we found that inescapable footshock produces robust c-Fos expression preferentially in CCK+ neurons of the BLA, and VTA. Furthermore, using Cre-dependent viral expression of mCherry in CCK-Cre mice, we demonstrated that CCK+ BLA pyramidal neurons and CCK+ VTA dopamine neurons project to the nucleus accumbens (NAcc). Finally, bath application of CCK-8 (the most common form of CCK in brain) onto nucleus accumbens slice preparations increases extracellular dopamine, as measured by the genetically encoded dopamine sensor dLight1.2.

Overall, these data suggests that multiple distinct CCK+ projection neurons to the nucleus accumbens may play distinct roles in maladaptive behaviors associated with aversive processing and traumatic experience.

Disclosures: O.P. Culver: None. **J. Parrilla-Carrero:** None. **T. Jhou:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.01

Topic: G.05. Mood Disorders

Support: National Institute of Child Health and Human Development R01HD067254

Title: Intrinsic Functional Connectivity in Childhood Predicts Somatic Symptom Change in Adolescence: A Four-Year Longitudinal Study

Authors: *C. SHAFFER[1], L. CUTTING[2], K. QUIGLEY[1], L. FELDMAN BARRETT[1], S. WHITFIELD-GABRIELI[1];
[1]Northeastern Univ., Boston, MA; [2]Vanderbilt Univ., Nashville, TN

Abstract: Identifying neural markers of prodromal mood disorder symptoms is crucial for understanding pathophysiological development and may inform intervention opportunities prior to disorder onset. Evidence suggests that the functional connectivity (FC) of brain regions implicated in visceromotor regulation and mood, such as the subgenual anterior cingulate cortex (sgACC), may be a viable marker of vulnerability in children, as it shows large-scale functional changes throughout development. Psychiatric research has linked aberrant connectivity patterns of the sgACC to the development of psychological and physical symptoms in children, adolescents, and adults. The present study hypothesized that FC patterns of the sgACC would predict changes in somatic complaints in a cohort of children at two time points. Ninety-three 7-year-old participants (39 female) who provided written informed consent and assent completed a baseline visit (T1) that included a resting-state MRI scan and a behavioral visit four years later (T2). Behavioral changes at T1 and T2 were assessed with the Somatic Complaints subscale of the Child Behavioral Checklist (CBCL-SOM). CBCL-SOM scores were not significantly different across age or gender and were not correlated with scan head motion. FC analysis was performed using a seed-driven approach; the sgACC seed was defined using prior exploration of sgACC connectivity in the developmental literature. A post-hoc analysis of covariance regressed CBCL-SOM change scores (T2 - T1) onto brain responses after controlling for initial severity at T1. The cluster-level threshold was set at $p < .05$ using false discovery rate correction for multiple comparisons, with voxel-wise t-value threshold of 3.40 (df = 89; $p < .001$). sgACC seed-to-voxel analysis revealed a cluster of voxels comprising the anterior and posterior inferior

temporal gyrus (ITG) and left middle temporal gyrus (MTG), which significantly predicted the four-year change from baseline on CBCL-SOM ($t_{92} = 4.72$, $p < 0.001$, β;=0.003). Specifically, greater connectivity between sgACC, ITG and MTG was associated with an increase in CBCL-SOM scores from T1 to T2. Results suggest FC patterns among regions implicated in visceromotor control and multimodal sensory integration may be a viable biomarker of change in somatic complaints across development. Somatic complaints are a key feature of adolescent mood disorder symptomatology. Our findings suggest that connectivity between the reported regions may contribute to the subjective sensory experience of the body for children. Thus, it may serve as a valuable biomarker of mood features that are unique to this developmental stage.

Disclosures: C. Shaffer: None. **L. Cutting:** None. **K. Quigley:** None. **L. Feldman Barrett:** None. **S. Whitfield-Gabrieli:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.02

Topic: G.05. Mood Disorders

Support: DOD W81 XWH-10-2-0181

Title: High frequency feedback processing as it relates to suicidal ideation and behavior

Authors: *A. PAVULURI[1], J. S. ELLIS[2], M. ARENSON[1], T. E. JOINER[3], N. B. SCHMIDT[3], E. M. BERNAT[1];
[1]Univ. of Maryland, College Park, MD; [2]Tech. Resources International, LLC, Bethesda, MD; [3]Florida State Univ., Tallahassee, FL

Abstract: ***Background.*** Suicidal ideation and behavior may have different etiological pathways, specifically related to feedback processing, that could be better understood by identifying relevant ERP components. Our previous findings in delta (0-3Hz) and theta (4-7Hz) bands indicated that individuals with suicidal behavior, but not ideation, showed heightened neurophysiological response across multiple ERP components to gambling feedback, particularly to loss stimuli, suggesting increased salience and evaluative processing for those with suicidal behavior. This study aimed to assess emerging loss and gain sensitive higher frequency effects in alpha (8-12Hz), beta 1 (12-20Hz), beta 2 (20-30Hz), and gamma (30-60Hz) in the same data, to assess a more comprehensive model of gain and loss sensitive systems in relation to suicidal ideation and behavior. ***Methods.*** 271 subjects (55.7% female; mean age=35.75, SD=16.07; 30.6% veterans) completed a questionnaire battery and a multi-task protocol, including a gambling feedback task (Gehring and Willoughby, 2002), while data was collected from a 96-channel EEG system. Exploratory factor analysis was conducted across 20 self-report suicide

items from relevant questionnaires and produced 2 factors, corresponding to suicidal ideation and suicidal behavior and explaining 60.05% of the variance. Time frequency principal component analysis identified a late slow-wave (1000-2000ms; LSW) parieto-occipital (POZ) measure in alpha, and a LSW bilateral fronto-temporal (F7-FT7-F8-FT8) measure in beta 1, beta 2, and gamma. ***Results.*** All measured frequencies, including previously measured delta and theta bands, were significantly related to suicidal behavior, and not to ideation, except alpha, which showed a relationship to ideation, and not to behavior. Beta 1-LSW, beta 2-LSW, and gamma-LSW were positively correlated with loss ($r=.24$, $p<.001$; $r=.22$, $p<.001$; $r=.21$, $p<.002$, respectively) and gain ($r=.24$, $p<.001$; $r=.21$, $p<.001$; $r=.19$, $p<.002$, respectively), but not with loss-gain differences. Alpha-LSW was positively correlated with loss ($r=.14$, $p<.02$) and gain ($r=.14$, $p<.03$), but not with loss-gain differences. Fisher's z values confirmed that the correlations were stronger for behavior than for ideation for all measured frequencies, except alpha. ***Discussion.*** Broadly, the higher frequency results indicate increased activity for behavior relative to ideation, which was not differentiated between gain and loss conditions, with the notable deviation of alpha relating to ideation, but not to behavior. Findings provide new information about differentiating neurophysiological characteristics relevant to suicide.

Disclosures: A. Pavuluri: None. **J.S. Ellis:** None. **M. Arenson:** None. **T.E. Joiner:** None. **N.B. Schmidt:** None. **E.M. Bernat:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.03

Topic: G.05. Mood Disorders

Title: Exploring the interaction between emotion regulation and inflammation on changes in episodic memory in older adults

Authors: *E. E. DICKER, S. L. LEAL, C. P. FAGUNDES, B. T. DENNY;
Psychological Sci., Rice Univ., Houston, TX

Abstract: Older adults commonly experience chronic, low grade inflammation as a result of age-related dysregulation. Thus, older adults typically have higher concentrations of circulating proinflammatory cytokines such as Interleukin (IL)-6 and (IL)-8. This predicts the development of physical decline, cognitive decline and Alzheimer's Disease and related dementias (ADRD). In contrast, older adults demonstrate relative emotional strengths compared to younger adults by engaging in more adaptive emotion regulation strategies such as cognitive reappraisal. Emotion regulation is associated with altered immune functioning, and such a link may be an important biological pathway by which interventions targeting affect may improve immune system-related cognitive outcomes. To investigate whether emotion regulation strategies differentially moderate

the effect of inflammatory cytokine levels on cognitive decline, this study used data from the Survey of Mid-life Development in the U.S. (MIDUS), which included measures of emotion regulation (cognitive reappraisal and suppression) and immune function (levels of IL-6 and IL-8). There were 189 adults (mean age = 53 years) who participated in both the MIDUS II and MIDUS III data collections (10 years apart). Regression models measured whether MIDUS II self-report of cognitive reappraisal moderated the effect of MIDUS II serum MSD IL-6, and IL-8 (pg/mL) levels on a composite z-score of episodic memory (immediate and delayed word recall task) at the MIDUS II timepoint and the MIDUS III timepoint 10 years later. Another set of regressions was conducted using the same predictors and criterion, except for MIDUS II self-report suppression, another emotion regulation strategy. All four models controlled for age, sex, BMI, smoking status, alcohol use status, race, years of education, and self-rated health. Only age and sex were significant predictors of MIDUS II episodic memory composite scores. Neither emotion regulation strategy nor either pro-inflammatory cytokine levels predicted memory. However, MIDUS II levels of IL-8 was a significant positive predictor of episodic memory scores 10 years later. Self-reported cognitive reappraisal predicted higher episodic memory scores at the MIDUS III timepoint. MIDUS II level of education, smoking status, and alcohol status also became significant predictors of episodic memory scores 10 years later in expected directions. These findings indicate that inflammation and emotion regulation strategies may be linked to age-related memory decline and could potentially provide a therapeutic target for interventions to improve immune system-related cognitive outcomes.

Disclosures: E.E. Dicker: None. **S.L. Leal:** None. **C.P. Fagundes:** None. **B.T. Denny:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.04

Topic: G.05. Mood Disorders

Title: The effect of emotion regulation on negative affect and emotional mnemonic discrimination

Authors: *B. HAYES, A. HARIKUMAR, L. FERGUSON, S. LEAL;
Rice Univ., Houston, TX

Abstract: Throughout life, stressful and negative experiences are often encountered. Emotion regulation strategies can be used to mitigate the negative effects of these events. These strategies can range from altering our view of the current situation to actively trying to suppress the negative emotions they convey. Depression is associated with general episodic memory deficits as well as a negativity bias, where negative experiences are better remembered compared to neutral or positive experiences. Recent findings have shown that the negativity bias may be

driven by enhanced detailed memory, which is supported by hippocampal pattern separation. Mnemonic discrimination tasks have been developed to examine the behavioral correlate of hippocampal pattern separation and have been shown to be sensitive in detecting memory alterations in depression. The current study aimed to investigate how emotion regulation strategies may be able to modulate negative affect and mnemonic discrimination in individuals with depressive symptoms. Participants completed an emotional mnemonic discrimination task that taxes hippocampal pattern separation that included negative and neutral images, in which they rated their negative feelings towards each image during encoding and retrieval. During the first half of encoding, participants simply viewed the images and rated their negative affect towards the images. During the second half of encoding, participants were either trained to apply an emotion regulation strategy (distancing) and rate their negative affect or were simply asked to rate their negative affect as they did in the first half, applying no emotion regulation strategy (controls). Participants completed a battery of questionnaires including the Beck Depression Inventory-II to measure depressive symptoms. During retrieval, participants were shown the same images (targets), brand new images (foils), or similar but not identical images to those shown during encoding (lures). These lure stimuli are critical for taxing hippocampal pattern separation. We found that the use of an emotion regulation strategy (distancing) was successful in decreasing negative feelings towards negative stimuli (both during encoding and retrieval) in healthy and depressed individuals. Further, we found that applying distancing during encoding impacted memory, resulting in a decrease in the negativity bias in depressed individuals and an increase in neutral memory. This suggests that applying emotion regulation strategies may be an effective way to alter mood and memory in depression.

Disclosures: B. Hayes: None. **A. Harikumar:** None. **L. Ferguson:** None. **S. Leal:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.05

Topic: G.05. Mood Disorders

Title: Development of a face-name mnemonic discrimination task

Authors: *R. MANNION, A. PENA, A. HARIKUMAR, S. LEAL;
Rice Univ., Houston, TX

Abstract: Face-name associative memory encodes information about the relationship between faces and names, allowing us to put faces to names. Difficulty remembering faces is a common struggle for many people, especially as one ages. For example, if you meet someone with a beard one day and a week later see them again but they shaved their beard, it may be difficult to recognize them due to subtle differences in their facial features. Further, it can also be

challenging to remember people's names, especially when they sound phonetically similar to other names (e.g. Lauren and Laurel). Subtle changes in appearance, common facial characteristics across individuals, and similarity between names contribute to the struggle of remembering face-name associations. Computational models suggest the hippocampus performs two key computations: first, reducing interference among similar experiences by using non-overlapping representations (pattern separation); second, filling-in incomplete or degraded representations based on those previously stored (pattern completion). While prior work has examined face-name associative memory, it has not investigated memory for face-name stimuli that have a high degree of similarity in an ecologically valid context. To this end, we developed a face-name associative memory task where facial similarity (same or similar person) and name similarity (same or similar name) were varied. In addition, the race, gender, and emotional valence of the stimuli were evenly distributed among targets (i.e., repeated items), lures (i.e., similar but not identical items), and foils (i.e., new items). Participants were shown a series of faces paired with names during encoding. During retrieval, participants were first shown just the faces (repeated faces, lure faces, and foil faces) followed by a second memory test of faces paired with names (repeat names, lure names, and foil names). We found that memory for face-name associations was reduced for lures compared to targets. This suggests that the inclusion of faces and names that share overlapping features in a memory task may provide a more sensitive measure of face-name associative memory functioning as well as establish the underlying neural mechanisms that support face-name associative computations.

Disclosures: R. Mannion: None. **A. Pena:** None. **A. Harikumar:** None. **S. Leal:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.06

Topic: G.05. Mood Disorders

Title: The effect of antidepressants on emotional mnemonic discrimination

Authors: *T. PHILLIPS, R. VAS, A. HARIKUMAR, L. FERGUSON, S. LEAL;
Rice Univ., Houston, TX

Abstract: Depression is associated with general memory impairment as well as a negativity bias, where negative experiences are better remembered compared to neutral or positive experiences. One potential mechanism underlying this negativity bias may be pattern separation, a hippocampal computation that processes similar experiences using non-overlapping representations. Individuals with depression show reduced hippocampal volume and enhancement of negative mnemonic discrimination, the behavioral correlate of hippocampal pattern separation. Rodent studies have shown that antidepressants can reverse hippocampal

volume reduction and restore normal memory functioning, but these effects have not yet been tested in humans. One caveat to consider from human studies of antidepressants is that more than half of all depressed patients do not respond to antidepressants and must try different types until they find one that works for them. The current study investigated the effect of antidepressant use and their perceived effectiveness on an emotional mnemonic discrimination task. Depressed young adults who were taking antidepressants performed a computerized emotional mnemonic discrimination task that taxes hippocampal pattern separation. During encoding, participants were shown positive, negative, or neutral images. After a short delay, participants were then tested on their memory for the images they saw during encoding with the same images they saw during encoding (targets), brand new images (foils), or similar but not identical images to ones they saw during encoding (lures). Participants reported their depressive symptoms prior to taking antidepressants as well as their current depressive symptoms, where we calculated a change score to determine how effective participants felt the antidepressants were in reducing their symptoms. We found that individuals taking antidepressants who reported a greater improvement in their symptoms showed a reduction in negative lure discrimination and enhanced neutral lure discrimination compared to those who were taking antidepressants with no improvement in symptoms. This suggests that antidepressants may be beneficial in alleviating mood symptoms as well as reducing the negativity bias and improving memory.

Disclosures: T. Phillips: None. **R. Vas:** None. **A. Harikumar:** None. **L. Ferguson:** None. **S. Leal:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.07

Topic: G.05. Mood Disorders

Title: The effect of current perceived stress on emotional mnemonic discrimination

Authors: *R. VAS, T. PHILLIPS, A. HARIKUMAR, L. FERGUSON, S. LEAL;
Rice Univ., Houston, TX

Abstract: Stress can have profound impacts on memory. The hippocampus, a brain region involved in memory, can perform a key computation called pattern separation that allows for the orthogonalization between similar events. Previous research has examined the impact of stress on hippocampal pattern separation by inducing stress in the laboratory or by examining chronic stress (e.g. depression); however, little is known about how natural variation in current perceived stress in healthy young adults may impact memory. Here, we examined the impact of current perceived stress on an emotional mnemonic discrimination task that taxes hippocampal pattern separation. Participants (ages 18-35) reported their current level of perceived stress on a

modified version of the Perceived Stress Scale (PSS) that asked participants about their current level of stress as well as stress over the past month. We used this measure to split participants into low and high current perceived stress groups. After administration of the PSS, participants performed an emotional mnemonic discrimination task. During encoding, participants viewed scene stimuli, which they rated as positive, negative, or neutral. After a short delay, their memory was tested for the same exact images they saw during encoding (targets), brand new scenes (foils), and similar but not identical scenes to ones they saw during encoding (lures). We found impaired lure discrimination in young adults with higher current perceived stress compared to those with lower current stress levels, especially for positive stimuli. These results suggest current perceived stress greatly impacts emotional memory, especially for positive experiences.

Disclosures: R. Vas: None. **T. Phillips:** None. **A. Harikumar:** None. **L. Ferguson:** None. **S. Leal:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.08

Topic: G.05. Mood Disorders

Title: Examining the effect of retirement and cognitive activity on mnemonic discrimination

Authors: *L. A. FERGUSON, A. HARIKUMAR, S. L. LEAL;
Rice Univ., Houston, TX

Abstract: Aging is associated with memory decline, especially for experiences that have many overlapping features. The hippocampus, a brain region involved in memory, can perform a key computation called pattern separation to reduce interference across similar experiences. Older adults are susceptible to interference and show hippocampal pattern separation impairment. However, other factors may influence the degree to which memory becomes impaired with age. Retirement may result in a significant change in one's life such that a transition from life in the workforce to leisure time at home may negatively impact memory. The reduction in cognitive activity that accompanies retirement could potentially reduce cognitive reserve in aging. In the current study, we examined how retirement status and cognitive activity impacted performance on a mnemonic discrimination task that taxes hippocampal pattern separation. We examined general memory performance (target recognition) as well as detailed memory performance (mnemonic discrimination). Preliminary evidence suggests that retired older adults have worse mnemonic discrimination performance compared to working older adults. There were no group differences for target recognition, suggesting mnemonic discrimination may be a more sensitive measure of decline associated with retirement. Further, age and level of cognitive activity

significantly predicted mnemonic discrimination performance. This suggests that retirement status may impact memory performance and could contribute to age-related memory decline. Further, older adults may be able to maintain their cognitive reserve through educational and cognitively stimulating activities.

Disclosures: L.A. Ferguson: None. **A. Harikumar:** None. **S.L. Leal:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.09

Topic: G.05. Mood Disorders

Support: Notre Dame-Mexico Research Collaboration Grant
25974 CONACyT graduate fellowship

Title: Test Re-test reliability of resting heart rate variability in undergraduate and graduate students from public and private universities in northwest Mexico

Authors: *N. CORRAL-FRIAS[1], J. L. ESPINOZA-ROMERO[2], M. FRIAS-ARMENTA[2], J. F. ESPINOZA[2], D. B. WATSON[3];
[1]Univ. De Sonora, Hermosillo, Mexico; [2]Univ. de Sonora, Hermosillo, Mexico; [3]Univ. of Notre Dame, Notre Dame, IN

Abstract: Heart rate variability (HRV) is the fluctuation in the interval between heart beats reflecting heart-brain interactions, which help in the adaptation to environmental and psychological challenges. Following the recent push to establish neurobiological markers of psychiatric illness for improved nosology, HRV has been used as a transdiagnostic marker. Despite its promising utility in the field of psychopathology, efforts to improve research reproducibility, and generalizability have only recently emerged in this field. Regardless of efforts to increase generalizability there is a dearth of HRV research in Latin American samples. Moreover, there is limited research determining the test re-test reliability of various HRV measures and methods. Using HRV data (5 minutes during rest) from 3 visits during an academic year we calculated test-retest reliability in undergraduate and graduate students from various public and private universities in a city in northwest Mexico (n = 236). Participants also completed self-report personality and mood and anxiety questionnaires. We found that HRV measures demonstrated time consistency reliability and were associated with weight and BMI. However, we did not find any consistent associations with mood and anxiety disorder symptoms. Our results suggest that HRV is reliable over time and is associated with biometric data. However, we did not find links to psychiatric symptoms. Future studies should include larger community samples to test the generalizability and reproducibility of our results.

Disclosures: N. Corral-Frias: None. **J.L. Espinoza-Romero:** None. **M. Frias-Armenta:** None. **J.F. Espinoza:** None. **D.B. Watson:** None.

Poster

P698. Human Imaging and Behavioral Studies: Mental Wellbeing

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P698.10

Topic: G.05. Mood Disorders

Support: R01MH101497

Title: Dorsal anterior cingulate cortex, anteromedial thalamus, and anterior insula effective connectivity during threat anticipation in individual with internalizing psychopathology

Authors: *M. RADOMAN[1], S. M. GORKA[2], K. L. PHAN[2], O. A. AJILORE[1];
[1]Univ. of Illinois at Chicago, Chicago, IL; [2]Ohio State Univ., Columbus, OH

Abstract: Converging lines of evidence suggest that heightened responses to unpredictable threat may be an important neurobiological marker of internalizing psychopathology. Prior data also indicate that aversive responding to uncertainty may be mediated by hyperactivation of several brain regions within the frontolimbic circuitry, namely the anterior insula (AIC), anteromedial thalamus, and dorsal anterior cingulate cortex (dACC). The present study aimed to understand how these three regions function as a network during U-threat and predictable threat (P-threat) anticipation in adults with internalizing psychopathologies. Towards this goal, functional magnetic resonance imaging (fMRI) and dynamic causal modeling (DCM) were used to examine effective connectivity among these three nodes in 22 healthy controls and 49 patients with various comorbid mood (depression and anxiety) disorders. During fMRI, participants completed a version of the well-validated No-Predictable-Unpredictable (NPU) paradigm. Six connectivity models, based on anatomic connections, were entered into Parametric Empirical Bayes (PEB) analysis to examine group-level differences in effective connectivity parameters between controls and patients. We further applied leave-one-out-cross-validation to evaluate the hypotheses about whether the effect size of connections identified in the PEB analysis was large enough to predict a participant's group (patient vs. control) from their neural response. The study yielded two main findings: (1) dACC to AIC connectivities during both P-threat and U-threat emerged as important group-difference parameters, but (2) only the effect size of dACC to AIC connectivity during U-threat was large enough to predict a participant's group. This study is the first to demonstrate in humans the key role of dACC projections to AIC in support of "top-down" threat processing. Further research efforts are needed to corroborate and expand the present findings.

Disclosures: M. Radoman: None. **S.M. Gorka:** None. **K.L. Phan:** None. **O.A. Ajilore:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH award 2R21NS102652
NIH award R01NS037112

Title: Genetic targeting of RGS14 to the nuclei of neurons induces sex-specific behavioral effects of cocaine in mice

Authors: *S. BRAMLETT[1], S. FOSTER[3], D. LUSTBERG[2], N. HARBIN[1], D. WEINSHENKER[4], J. HEPLER[5];
[2]Mol. & Systems Pharmacol., [1]Emory Univ., Atlanta, GA; [3]Dept. of Human Genet., Emory Univ. Sch. of Med., Atlanta, GA; [4]Dept Human Genet., Emory Univ. Sch. Med., Atlanta, GA; [5]Emory Universitty Sch. of Med., Atlanta, GA

Abstract: Psychostimulant addiction is a major public health problem that currently lacks any U.S. Food and Drug Administration-approved medications for treatment. Psychostimulants such as cocaine (COC) induce hyperdopaminergic signaling within the mesolimbic reward pathway to usurp the neural mechanisms of motivational drive. Regulator of G protein Signaling 14 (RGS14) is a multifunctional scaffolding protein in brain that is best understood for its role in suppressing synaptic plasticity and learning and memory in hippocampal area CA2. We have proposed that RGS14 regulates signaling in the nucleus accumbens in a similar manner to oppose the maladaptive engagement of learning and memory that forms the basis of addiction. Herein we examine the relationship of RGS14 to molecular and behavioral correlates of COC addiction, as well as the role that RGS14 subcellular localization plays in mediating the effects of COC. Two genetically manipulated mouse lines are used to accomplish these goals: one strain in which RGS14 is functionally deleted (RGS14-KO) and another in which RGS14 is confined to the nucleus by CRISPR/Cas9 introduction of a naturally occurring variant found in humans (RGS14-L504R). Behavioral tasks include acute locomotor response and locomotor sensitization to COC. Immunofluorescence co-staining of RGS14 and addiction-linked signaling proteins in striatal sections of mice treated with COC acutely and chronically are used to reveal intracellular mechanisms. Our analysis revealed extensive colocalization of RGS14 with D1 and D2 dopamine receptors in nucleus accumbens core and shell, supporting RGS14 regulation of reward pathway neurons. Furthermore, we found that subjects in which RGS14 is restricted to the nucleus (RGS14-L504R) show enhanced sensitivity to the locomotor activating effects of COC. This effect is demonstrated by a leftward shift in the dose-response curve and is driven by RGS14-L504R males. A different relationship is observed in the RGS14-KO group, which show

an augmented maximal response of COC-induced locomotor activation in a sex-independent manner. These results reveal exciting new information regarding the diverse roles that RGS14 can fill in respect to COC action depending on where it is localized in the cell, which may provide an avenue to much-needed therapeutics for psychostimulant addiction.

Disclosures: S. Bramlett: None. **S. Foster:** None. **D. Lustberg:** None. **N. Harbin:** None. **D. Weinshenker:** None. **J. Hepler:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R01DA047441-02

Title: Sex differences in the role of GILZ underlying formation and maintenance of drug-associated memory

Authors: *J. ROUNDS, E. KRAMÁR, M. WOOD;
Univ. of California, Irvine, Irvine, CA

Abstract: It is well established that stress can modulate interactions between drugs of abuse and memory processes in the brain. The conditions of a stressful experience can differentially impair or facilitate memory, and in certain situations may reinstate drug-seeking behaviors. However, the mechanisms by which stress leads to stable changes in neuronal function underlying long-term behavioral adaptations remain poorly understood. Here, we examined the role of a candidate X-linked gene (glucocorticoid-induced leucine zipper, GILZ) in regulating long-term drug-associated memory processes within the mouse nucleus accumbens (NAc) and ventral tegmental area (VTA). We found sex-specific patterns of baseline GILZ expression in both the NAc and VTA, where mRNA splice variants coding for the various GILZ protein isoforms were differentially expressed between adult male and female mice. The differential expression of these GILZ isoforms (known to regulate transcriptional activation as well as the integration of key signaling cascades involved in memory) thus suggests sex differences in the role of GILZ in reward-related regions. To determine whether GILZ expression is necessary for the formation of drug-associated memory, males and females were infused with a site-specific siRNA (targeted to either the NAc or VTA) to knockdown all splice variants of GILZ prior to training in a cocaine-conditioned place preference (CPP) protocol. We found that VTA-GILZ knockdown significantly blunted cocaine-CPP acquisition in males but not females in a dose-dependent manner. In addition, we found that intra-NAc knockdown of GILZ attenuates long-term potentiation (LTP, a key measure of synaptic involvement in memory formation) in males.

Together, these data suggest a sex-specific role of GILZ in contributing to stable changes in neuronal function underlying reward-related memory processes.

Disclosures: J. Rounds: None. **E. Kramár:** None. **M. Wood:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA025646
Washington State Initiative 171 research funds administered through the WSU Alcohol and Drug Abuse Research program

Title: Corticotropin-releasing factor receptor 1 in the basolateral amygdala regulates cocaine-memory reconsolidation in male and female rats

Authors: *J. L. RITCHIE, J. L. WALTERS, J. M. C. GALLIOU, R. J. CHRISTIAN, S. QI, M. I. SAVENKOVA, C. K. IBARRA, S. R. GROGAN, R. A. FUCHS;
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Abstract: Exposure to drug-associated environmental stimuli can lead to the retrieval of powerful drug memories and elicit drug craving and relapse in individuals with substance use disorders. Drug memories can become destabilized upon retrieval, requiring memory reconsolidation to maintain their strength over time. The basolateral amygdala (BLA), a critical site of memory reconsolidation, robustly expresses corticotropin releasing factor receptor 1 (CRFR1). Thus, we evaluated whether BLA CRFR1 signaling is sufficient or necessary for cocaine-memory reconsolidation in Exp 1. Male and female Sprague Dawley rats self-administered cocaine in a distinct context for 10 days then received extinction training in a different context for 7 days. Next, the rats were exposed to the cocaine-paired context to reactivate cocaine memories and received bilateral intra-BLA vehicle, CRFR1 antagonist (antalarmin 500 ng/hemisphere), or exogenous CRF (0.2, 30, or 500 ng/hemisphere) infusions immediately, or 6 h (control), after memory reactivation. Drug-seeking behavior was assessed in the cocaine-paired context 3 d later to evaluate cocaine-memory strength. Intra-BLA CRFR1 antagonism immediately, but not 6 h, after memory reactivation attenuated drug-seeking behavior at test independent of sex. Conversely, intra-BLA CRF dose-dependently enhanced drug-seeking behavior selectively in females. In Exp 2, we aimed to identify the source of CRF in the BLA during cocaine-memory reconsolidation. Rats received intra-BLA infusions of Retrobeads^{TM} prior to behavioral training. Brain tissue was collected 2 h after memory reactivation (i.e., during reconsolidation) or home cage stay. Retrobeads^{TM}, CRF

immunoreactivity (IR), and c-Fos-IR (marker of neuronal activation) were visualized. BLA-projecting CRF-IR neurons in the anterior cingulate cortex, prelimbic cortex, and dorsal raphe, but not in the infralimbic cortex or anterior insular cortex, exhibited greater c-Fos expression after cocaine-memory reactivation relative to home cage stay. These findings indicate that CRF signaling in the BLA is necessary for cocaine-memory reconsolidation, and distinct extra-hypothalamic CRF circuits may be recruited to maintain cocaine-memory strength during reconsolidation.

Disclosures: J.L. Ritchie: None. **J.L. Walters:** None. **J.M.C. Galliou:** None. **R.J. Christian:** None. **S. Qi:** None. **M.I. Savenkova:** None. **C.K. Ibarra:** None. **S.R. Grogan:** None. **R.A. Fuchs:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.04

Topic: G.09. Drugs of Abuse and Addiction

Support: UH3 NS096833
R01 DA049544

Title: Corticotrophin Releasing Factor Receptor 2 in the basolateral amygdala enables Nonmuscle Myosin II-mediated disruption of methamphetamine-associated memory

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Abstract: Substance use disorders (SUD) are perpetuated by associative memories that induce motivation to seek drug. We previously reported that inhibition of the actin motor ATPase nonmuscle myosin II (NMII) with the small molecule inhibitor blebbistatin (blebb), results in actin depolymerization and dendritic spine loss in the basolateral amygdala (BLA) and a retrieval-independent disruption of methamphetamine (METH)-associated memories and METH seeking. The effect is unique to BLA and specific to METH, as it does not interfere with other associative memories for foot shock, food reward or many other commonly abused drugs, including cocaine (COC). To investigate the source(s) of this selectivity, we used RNA-seq following conditioned place preference (CPP) conditioning to determine transcriptional differences between METH- and COC-associated memories. One gene selectively upregulated in METH-treated mice in BLA, but not hippocampus or nucleus accumbens, was *crhr2*, which encodes Corticotrophin Releasing Factor receptor 2 (CRF2). Besides its role in the stress

response, its ligand, CRF, is released in BLA following COC or METH administration, and CRF receptor 1, but not CRF2, is involved in COC-associated memory. Interestingly, CRF2 has been linked to NMII regulation outside the CNS, but its role and potential interaction with NMII in METH-associated memories was unknown. To test for this interaction, we first established the impact of CRF2 inhibition after METH CPP conditioning. Mice received intra-BLA infusions of vehicle or the CRF2 antagonist Astressin-2B (AS2B) after the final METH conditioning session. CRF2 inhibition was not sufficient to disrupt the memory, as both groups expressed a METH CPP when tested 48hr later. A second experiment established that, unlike AS2B, NMII inhibition with blebb (IP) shortly after the last METH conditioning session disrupted METH CPP expression. Taken together, these results enabled examination of the interaction between CRF2 and NMII. For this, mice received intra-BLA infusions of vehicle or AS2B after the last conditioning session, followed by blebb (IP) shortly after. METH CPP was disrupted in mice treated with vehicle and blebb, replicating our previous results. However, METH-associated memory was protected in mice that received AS2B and blebb. These results suggest that CRF2 is required in combination with NMII activity in BLA to render METH-associated memories and seeking-like behavior selectively vulnerable to NMII inhibition in the absence of retrieval. Indeed, CRF2 may represent a general target for destabilizing memory via (yet to be identified) downstream effects on NMII.

Disclosures: M. Hafenbreidel: None. **S.B. Briggs:** None. **M. Arza:** None. **A. Tiller:** None. **A.B. Hall:** None. **L. Lin:** None. **S. Khan:** None. **S.E. Sillivan:** None. **S. Pandey:** None. **M.D. Cameron:** None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council of Canada

Title: Role of dopamine receptors in conditioned memory modulation

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Abstract: Exposure to conditioned stimuli (CS) predictive of foot-shock enhance memory consolidation, a process of memory stabilization that is central to learning. There is evidence that dopamine D2 and D3 receptors modulate several responses to CSs, including freezing and step-through passive avoidance. Therefore, we employed a signalled active avoidance procedure (8 days; 30 trials/day; 0.8 mA) and spontaneous object recognition (SOR) task to examine whether

these DA receptors are involved in the memory enhancing action of a CS predictive of foot-shock. As such, male Sprague-Dawley rats were exposed to the avoidance CS immediately following the sample phase of the SOR task. Immediate post-sample exposure to the avoidance CS in the absence of shock enhanced object memory, and this effect was dose-dependently blocked by the beta-adrenergic receptor antagonist propranolol (10 and 20 mg/kg), thus confirming that this conditioned effect on object recognition is indeed facilitated by a modulation of consolidation. We then tested the D2 receptor antagonist pimozide (0.2 and 0.6 mg/kg) and D3 receptor antagonist NGB-2904 (0.1 and 5 mg/kg), both of which dose-dependently blocked the effect of post-sample CS exposure on object memory, while having no effect on avoidance behaviour. Overall, these results indicate an important role for DA D2 and D3 receptors in conditioned enhancement of memory consolidation.

Disclosures: T. Lapointe: None. **F. Leri:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.06

Topic: G.09. Drugs of Abuse and Addiction

Support: National Science and Engineering Research Counsel of Canada

Title: Role of perirhinal cortical nicotinic receptors in memory modulation by nicotine, cocaine, and their conditioned stimuli

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Abstract: It has been recently suggested that drug conditioned stimuli (CS) activate neurochemical systems of memory modulation that are directly activated by the drugs themselves. To test this hypothesis using two psychomotor stimulant drugs, nicotine and cocaine, male Sprague-Dailey rats received infusions of the nicotinic receptor antagonist mecamylamine (MEC) within the perirhinal cortex (PRh); a cortical region well known to be involved in consolidation object memory. To establish drug CSs, rats were confined for 2 h in a chamber (the CS+) after injections of 0.4 mg/kg nicotine or 20 mg/kg cocaine, and in another chamber (the CS−) after injections of vehicle. This was repeated over 10 days (5 drug/CS+ and 5 vehicle/CS− pairings in total). It was found that the memory enhancing action of post-sample 0.4 mg/kg nicotine was blocked by intra-PRh infusions of 10 and 30 μg/side MEC, and 30 μg/side MEC also blocked the memory enhancing effects of the nicotine CS. Interestingly, MEC did not block

the effects of cocaine, or the cocaine CS on object memory consolidation. These data indicate that a nicotine CS promotes memory consolidation by stimulating nicotine receptors in the PRh, an effect that is typically produced by nicotine itself. This cholinergic pathway, however, does not appear involved in memory modulation by CSs paired with other drugs.

Disclosures: M. Wolter: None. **N. Baidoo:** None. **K.A. Mitchnick:** None. **C. Wideman:** None. **B.D. Winters:** None. **F. Leri:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R00DA042102-04
NARSAD Young Investigator Reward

Title: Neuronal ensembles in the prelimbic cortex mediate initial cocaine-seeking behavior in rats.

Authors: *B. SORTMAN, S. RAKELA, K. SIBSON, B. CERCI, C. GOBIN, B. WARREN; Univ. of Florida, Gainesville, FL

Abstract: Neuronal ensembles in the medial prefrontal cortex (mPFC) mediate well-trained self-administration for both drugs and natural reinforcers. We have previously shown that mPFC ensembles drive food self-administration behavior after as few as one training session. Here, we test the hypothesis that neuronal ensembles within the prelimbic cortex (PrL) rapidly form to drive cocaine-seeking behavior after initiating cocaine self-administration. To test this hypothesis, we used the Daun02 inactivation procedure in Fos-lacZ transgenic rats to selectively ablate neuronal ensembles formed during initial cocaine-seeking behavior. We trained adult male and female Fos-LacZ rats to self-administer cocaine intravenously (0.75 mg/kg/infusion) for between 1 to 12 days, until rats met acquisition criteria (e.g., ≥30 active lever presses and ≥70% of all lever presses on the active lever). The day after each rat met behavioral acquisition we bilaterally microinfused either Daun02 or vehicle into the PrL (2µg/0.5µl/side) of rats 60 minutes after a 30-minute non-reinforcing cocaine seeking test (i.e., induction day). Forty-eight hours later we measured cocaine-seeking behavior in a 30-minute test under non-reinforced conditions. Rats that received Daun02 pressed the active lever significantly less than rats that received vehicle ($p<0.05$). We then extracted the brains to assess total β-galactosidase activity and found that β-galactosidase expression was significantly lower in Daun02-treated rats compared to vehicle-treated controls ($p<0.05$). These data suggest that neuronal ensembles in the PrL rapidly form to mediate cocaine self-administration after initial behavioral acquisition.

Disclosures: B. Sortman: None. **S. Rakela:** None. **K. Sibson:** None. **B. Cerci:** None. **C. Gobin:** None. **B. Warren:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant 5R00DA042102-04
Brain and Behavior Research Foundation NARSAD Young Investigator Grant

Title: Self-administration acquisition rate predicts locomotor, but not reward sensitivity to cocaine in rats

Authors: *S. RAKELA, B. SORTMAN, M. REEVES, C. GOBIN, B. WARREN;
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Abstract: Most people who initiate cocaine use do not develop Cocaine Use Disorder. Differences in susceptibility begin to emerge after first exposure to cocaine: some users discontinue cocaine self-administration after a single exposure, some use intermittently over a long-period of time, and others promptly develop a compulsive binge pattern. In rodents, individual differences in cocaine self-administration also emerge rapidly. Some rats rapidly acquire cocaine self-administration behaviors, while others require multiple training sessions. Whether these differences reflect differences in cocaine sensitivity, motivation, or capacity to acquire the behavior remain unclear. Here, we attempt to determine whether rate of cocaine acquisition predicts subsequent cocaine self-administration behavior, locomotor sensitivity to cocaine, or seeking following two weeks of abstinence. We first evaluated 14 male Sprague-Dawley rats in an open field test to gauge initial locomotion and anxiety-like behavior. Next, we subjected rats to daily 3h cocaine (0.75mg/kg/infusion) self-administration sessions until rats met acquisition criteria (≥30 active lever presses, and ≥75% responding on the active lever). Upon meeting acquisition criteria, we trained rats for 10 additional sessions to ensure all had equal opportunity to self-administer cocaine. Following self-administration training, we measured performance in a progressive ratio test, locomotor sensitivity to cocaine, and lever pressing in a non-reinforced cocaine seeking test after 2w of forced abstinence. Early Learners exhibited significantly less locomotion following an intraperitoneal injection of cocaine than Late Learners, but did not differ in any other behavioral parameter examined. These results suggest that time taken to acquire cocaine self-administration behavior is not dependent on factors such as motivation for or exposure to cocaine, but rather may be linked to physiological factors that predispose rats to learn an operant task, such as cocaine sensitivity.

Disclosures: S. Rakela: None. **B. Sortman:** None. **M. Reeves:** None. **C. Gobin:** None. **B. Warren:** None.

Poster

P733. Learning and Memory in Addiction I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P733.09

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA033641
T32DA028874

Title: Paternal Cocaine Self-Administration Enhances Fear-Related Phenotypes and Suppresses Synaptic Activity only in Male Offspring

Authors: *M. T. RICH, S. E. SWINFORD-JACKSON, S. T. MANKAME, S. J. WOROBEY, R. C. PIERCE;
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Abstract: Paternal exposure to cocaine induces epigenetic modifications that directly influence synaptic plasticity as well as memory- and reward-related behaviors. Given the high degree of comorbidity between substance use disorders and mental health illnesses, such as post-traumatic stress disorder (PTSD), we examined whether cocaine self-administration in male rats would produce fear-associated behavioral effects in drug-naïve progeny. Adult offspring were subjected to cue-dependent fear conditioning, followed by cue extinction. We observed no differences in fear-related freezing behavior during conditioning. However, cocaine-sired male offspring exhibited deficits in extinction as well as increased freezing upon retrieval of the extinction memory, indicative of enhanced and persistent fear-associated memory relative to saline-sired controls. Consistent with our previous results with other behavioral models, we found no differences in fear-related behaviors in female offspring. To determine a physiological correlate for these behavioral results, we next performed patch clamp recordings in the amygdala, a brain region that is highly involved in the encoding of emotionally salient memories. Despite no differences in baseline physiological measures, we found deficits in long-term potentiation (LTP) at cortico-amygdala synapses in male, but not female, offspring of cocaine-exposed sires. Prior research heavily implicates this circuit's involvement in fear extinction, suggesting that the resilience to fear memory extinction observed in cocaine-sired male progeny may be due to inherited deficiencies in genes that govern LTP induction. Ongoing studies are therefore investigating whether paternal cocaine exposure affects the expression of several known candidate genes that play a role in synaptic plasticity.

Disclosures: M.T. Rich: None. **S.E. Swinford-Jackson:** None. **S.T. Mankame:** None. **S.J. Worobey:** None. **R.C. Pierce:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P20GM103642
NSF Grant 1736026
NIH Grant 2R25NS080687
5R25GM061151-19
NIH Grant P20GM103475
NSF Grant 1633184
NSF Grant 2131647

Title: The role of histone acetyltransferase activity in alcohol-induced neuroadaptations

Authors: *A. MONTES, C. DASTA-CRUZ, L. RAMOS-RODRIGUEZ, C. DEL VALLE-COLON, M. KUCHIBHOTLA, J. L. AGOSTO, A. GHEZZI;
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Abstract: Excessive alcohol consumption is one of the most prevalent disorders among individuals in our society that affects adults, teens, and children that are born from alcoholic parents. Alcohol consumption leads to an homeostatic imbalance in the organism, followed by the development of adaptations in the brain leading to alcohol tolerance and physiological dependence. Recently, epigenetic mechanisms that alter gene expression and chromatin states have shown to be involved behind these alcohol-induced adaptations. However, much of the epigenetic mechanisms that lead to alcohol addiction are still unknown. In this study, we aim to determine the mechanism of histone modification upon alcohol exposure. To determine the effects of Tip60, a histone acetyltransferase, in alcohol tolerance, we used the model organism Drosophila melanogaster and the UAS-Gal4 system, to knock-down gene expression through RNAi of Tip60 in neurons (ELAV-GAL4/UAS-Tip60-RNAi). Using an activity monitor, we measured the difference in the rate of sedation upon alcohol vapor exposure of aged-matched, female ELAV-GAL4/UAS-Tip60-RNAi and control (ELAV-GAL4/UAS-GFP) adult flies. We found that Tip60 knock-down flies did not show a significant increase in sedation time between exposure to alcohol compared to control flies. These results suggest that Tip60 is involved in the development of alcohol tolerance. We also performed an RNA-sequencing of the whole brain of the aforementioned transgenic Drosophila to examine the transcriptional changes of alcohol sedated flies compared to mock-sedated flies after one day of exposure. This study reveals

differential expression of genes in neurons from ELAV-GAL4/UAS-Tip60-RNAi flies exposed to alcohol compared to mock-sedated flies, and for sedated and mock-sedated ELAV-GAL4/UAS-GFP flies, respectively. This suggests that alcohol exposure triggers significant transcription of ethanol-responsive genes, suggesting that complex gene networks might be involved in the development of alcohol tolerance. Collectively, our data can provide us new insights on how neurons adapt to alcohol through histone acetylation mechanisms.

Disclosures: A. Montes: None. **C. Dasta-Cruz:** None. **L. Ramos-Rodriguez:** None. **C. Del Valle-Colon:** None. **M. Kuchibhotla:** None. **J.L. Agosto:** None. **A. Ghezzi:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R25-NS080268706

Title: Molecular mechanisms of alcohol neuroadaptation in a Drosophila model

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Abstract: Alcohol consumption is known to induce cognitive impairments mainly affecting executive functions, episodic memory, and other capacities related to brain function. Nevertheless, the cellular and molecular mechanisms underlying such interactions are still unknown. Recent evidence has uncovered a similar interaction between ethanol exposure and cognitive function in the fruit fly, Drosophila melanogaster, which opens the way for molecular studies in a genetically tractable model system. Using olfactory conditioning assays where an odorant is used as a conditioned stimulus (CS) and is paired with a heat shock used as an unconditioned stimulus (US), it was shown that Drosophila larvae can learn to avoid the odor in future exposures. However,when the animals are exposed to a short acute dose of alcohol, they are no longer able to learn this association. Interestingly, larvae that have undergone prolonged chronic ethanol exposure seem to successfully avoid the odorant paired with the heat shock just as well as control ethanol-naive larvae, which is suggestive of ethanol-induced neuroadaptations. Our aim is to understand the genetic and cellular components responsible for this adaptation. For this, we employ RNA Sequencing technology to evaluate differences in gene expression in the brain of larvae chronically exposed to ethanol and in control larvae. With the knowledge

obtained from this study we could be able to understand ethanol's effect on learning and memory and gain an insight into how addiction may be contributing to damages in this behavior.

Disclosures: C. Irizarry-Hernandez: None. **N. Fuenzalida:** None. **A. Ghezzi:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA027768 (J.W.)
NIAAA U01AA025932 (J.W.)

Title: Cortical and Thalamic Inputs to D2-MSNs Distinctly Mediate Alcohol Seeking and Extinction

Authors: *T. TAN[1], A. BINETTE[1,2], W. WANG[1], Y. HUANG[1], Y. CHENG[1], W. PURVINES[3], X. XIE[1], B. HENDERSON[1], H. GANGAL[3], X. WANG[1], J. WANG[1,2];
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Abstract: The dorsal medial striatum (DMS), which plays an important role in alcohol use disorder (AUD), contains mainly dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons (MSNs). While the contribution of D1-MSNs to AUD is relatively clear, little is known about the role of D2-MSNs. Here, we explore the distinct roles of D2-MSNs in seeking and extinction of operant alcohol self-administration. A novel D2-Cre transgenic rat was crossed with a tdTomato reporter line. Histology study revealed the tdTomato-expressing striatal MSNs projected to the external globus pallidus (GPe), but not the substantia nigra pars reticularta (SNr). Slice electrophysiological studies found the firing activity of these fluorescent neurons was inhibited selectively by a D2R agonist but not D1R agonist, demonstrating the selective Cre expression in D2-MSNs. Interestingly, optogenetic excitation of DMS D2-MSNs during operant alcohol self-administration (SA) transiently reduced alcohol-seeking behavior, whereas optogenetic induction of cortico-striatal long-term potentiation (LTP) in these neurons produced significant and long-lasting suppression of alcohol-seeking behavior. Next, we examined the role of D2-MSNs in the extinction and discovered glutamatergic potentiation D2-MSNs after extinction training. Consistently, optogenetic inhibition of D2-MSN projections in the GPe prevented alcohol extinction and facilitated reinstatement to alcohol seeking. Retrograde (with rabies) and antegrade (with AAV1-Cre) tracing studies showed mono-synaptic afferent from the centromedian thalamic nucleus (CM) to D2-MSNs. Optogenetic inhibition of CM neurons or optogenetic induction of long-term depression (LTD) of CM inputs to DMS D2-MSNs,

prevented alcohol extinction. Taken together, our results suggest DMS D2-MSNs and cortical inputs to this neuronal type negatively regulate operant alcohol-seeking behavior, and thalamic inputs to DMS D2-MSNs positively regulate the extinction of alcohol SA. These results establish circuit and synaptic mechanisms of D2-MSNs in regulating alcohol-seeking and extinction, which may provide a therapeutic strategy in using these mechanisms to treat AUD.

Disclosures: T. Tan: None. **A. Binette:** None. **W. Wang:** None. **Y. Huang:** None. **Y. Cheng:** None. **W. Purvines:** None. **X. Xie:** None. **B. Henderson:** None. **H. Gangal:** None. **X. Wang:** None. **J. Wang:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P20GM103642
NIH Grant 2R25NS080687

Title: Neurophysiological correlates of alcohol tolerance in the Mushroom Body of Drosophila melanogaster

Authors: N. L. FUENZALIDA-URIBE, C. IRIZARRY-HERNANDEZ, I. A. DIAZ-NIEVES, *A. GHEZZI;
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Abstract: Alcohol exposure can generate a series of behavioral adaptations that lead to the development of alcohol tolerance and dependence. These adaptations are critical components in the development of alcohol addiction. However, the neural mechanisms by which these adaptations occur remain unresolved. Here, we use a Drosophila model to explore the neurophysiological correlates of alcohol tolerance in the Mushroom Body of the fly—a key neuropil in the brain. Flies develop tolerance to alcohol after a single exposure, and this phenotype is accompanied by a series of behavioral adaptations that remain for days. Since the Mushroom Body (MB) is an integration center of several sensory stimuli, we assessed its role in underlying ethanol tolerance. Using a functional imaging approach, we studied the neurophysiology of the MB calyx in response to ethanol. Flies expressing the calcium sensor protein GCamp6s in MB neurons were treated with ethanol vapor (~95%) until anesthetized (~40 minutes). Twenty-four hours later, we record the intracellular calcium dynamics in the MB calyx. Tolerant flies showed a decreased response to a short ethanol exposure (3 seconds) compared to control flies, suggesting this brain area is involved in the neurophysiological processing of ethanol tolerance. This reduction in neural signaling correlates with the changes

observed in Drosophila behaviors related to ethanol exposure, suggesting a possible neural substrate for the behavioral adaptation to alcohol during tolerance.

Disclosures: N.L. Fuenzalida-Uribe: None. **C. Irizarry-Hernandez:** None. **I.A. Diaz-Nieves:** None. **A. Ghezzi:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P20GM103642
NSF Grant 1736026
NSF Grant 1633184
NIH Grant 2R25NS080687
NIH Grant P20GM103475
NSF 2131647

Title: Pumilio -a translational regulator- as a modulator of alcohol tolerance via macrophages

Authors: *M. KUCHIBHOTLA, A. MONTES, E. W. O. ELIAS, J. A. RODRIGUEZ, J. AGOSTO, A. GHEZZI;
Biol., Univ. of Puerto Rico, Rio Piedras, San Juan, PR

Abstract: Alcoholism is the third leading cause of deaths. One of the key components in the development of alcoholism is the gradual increase in resistance to alcohol over repeated exposures. This adaptation is also known as tolerance, which is thought to involve many neural adaptations and plasticity within the brain leading to alcohol addiction. Alcoholism being a multifaceted disorder coupled with relapse, makes it harder for treatment. The Neuroimmune system is paving a new path in understanding the vital role of the innate immune system in the development of alcohol addiction and behaviors. Alcohol induced neuroinflammation and neurodegeneration at CNS is linked to both spiked systemic proinflammatory factors and mutations in these innate immune genes. Supporting the role of immunity and neuroimmune signaling in alcohol addiction. Therefore, there is a need for novel strategies and varied perspectives in treating alcoholism effectively. *Drosophila* studies have identified the RNA binding protein pumilio (pum) as a regulator of ethanol tolerance. Pum has also been linked to innate immunity and alcohol tolerance. Thus, the goal of this study is to investigate the role of pumilio in alcohol responses via macrophages, one of the key immune cells in the fly. To Determine how pum modifications within macrophages affect alcohol sensitivity and tolerance, we employed UAS/Gal4 binary system for targeted expression of pumilio RNAi transgene

precisely in hemocyte-specific driver line (hml-GAL4). We exposed age matched adult female progeny (hml-RNAipum and hml-GFP) to 50% ethanol and recorded sedation scores. We found that pum knockdown in hemocytes decreases ethanol tolerance without changing sensitivity. These findings suggest that hemocyte/brain communication contributes to alcohol responses and pum regulates crosstalk between hemocytes and brain by possibly altering these signaling. However, neuronal adaptations underlying this reduction in tolerance remain unknown. To understand those adaptations, transcriptome analysis of *Drosophila* whole brains was performed to survey genome wide expression profiles of hml-RNAi vs GFP flies after one exposure. Analysis of data is in the process. Our data indicate that Pumilio rather affects signaling between peripheral macrophages and the brain leading to neuronal plasticity. Mechanisms underlying these neuroimmune signaling pathways regulated by pumilio are worth exploring and can deepen our knowledge about the role of the immune system in alcoholism. Together these findings can spot macrophages as a possible potential novel therapeutic target in treating alcoholism.

Disclosures: M. Kuchibhotla: None. **A. Montes:** None. **E.W.O. Elias:** None. **J.A. Rodriguez:** None. **J. Agosto:** None. **A. Ghezzi:** None.

Poster

P739. Alcohol: Circuitry and Neurophysiology of Addiction II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:00 PM - 4:00 PM

Program #/Poster #: P739.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Regulated expression of the $GABA_A$receptor alpha2 subunit modulates the development of Alcohol Use Disorder

Authors: *J. BARKER, R. HINES, D. HINES;
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Abstract: The development of Alcohol Use Disorder (AUD) involves a combination of environmental and biological factors that lead to changes in both gene expression and function, dysregulation of proteins translated from mRNA, and synaptic plasticity. Both the timing and specific mechanisms that contribute to developing AUD remain unclear. Multiple studies have indicated an increase in expression of mRNA encoding the α2 subunit of the $GABA_A$ receptor following exposure to alcohol and other substances of abuse. In addition, genome-wide association studies of families with multiple alcoholics have shown single nucleotide polymorphisms in the GABRA2 gene, which codes for the α2 subunit. We find that the α2 subunit is highly expressed in the nucleus accumbens (NAc) on dopamine receptor D1 expressing cells. We also find that NAc expression of α2 subunit protein is increased upon voluntary access to alcohol. Mice with saturated α2 expression do not develop place preference for alcohol, and also do not increase voluntary consumption of alcohol in an intermittent access

paradigm. Our research suggests that regulated expression of the α2 subunit of the $GABA_A$ receptor is critical for the development of AUD. We are currently investigating the mechanisms that regulate α2 expression in the NAc during exposure to alcohol, including looking at proteins that control translation of α2 mRNA. These studies are expected to increase our understanding of the mechanisms underlying AUD, as well as promote the development of more specific therapies for treatment. These studies may also provide a potential target for early detection of those at-risk for AUD.

Disclosures: J. Barker: None. **R. Hines:** None. **D. Hines:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.01

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH124695-01
BBRF NARSAD Young Investigator Award
PITTSBURGH FOUNDATION Rising Star Award

Title: Striatal dopamine dynamics in freely moving postnatal $Shank3^{-/-}$ mice

Authors: *M. JANECEK[1,2], R. T. PEIXOTO[1,2];
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Abstract: Dysregulation of dopamine (DA) signaling in striatal circuits has emerged as a potential convergent mechanism in autism spectrum disorders (ASD). However, whereas striatal DA signaling has been well characterized during adulthood, little is known about its regulation during postnatal development. This knowledge gap persists due to technical limitations associated with early postnatal in vivo optical and electrophysiological measurements that are complicated by the rapid growth of connective and neural tissue. Of particular interest is the dorsomedial striatum (DMS), critical for learning and execution of motor actions, which may undergo a dopamine-sensitive maturation period in the first three postnatal weeks. Previously, we observed that mice with mutations in the SHANK3 gene (Shank3-/-), a highly penetrant risk factor for ASD, exhibit hyperactivity and increased corticostriatal connectivity of DMS during postnatal days (P) 10-20. Within this timeframe, Shank3-/- mice begin to present compulsive-like overgrooming and motor deficits, coinciding with pronounced psychomotor deficits in infants with ASD. These structural and behavioral phenotypes might underlie or arise from abnormal DA signaling. To address this question, we developed a fiber photometry protocol to measure striatal DA dynamics in freely moving ~P15 pups using the genetically encoded dopamine sensor dLight. We find that 14 days of expression suffice to induce robust dLight

expression in the striatum and demonstrate that the ~P14 skull can support a chronic fiber optic implant and a cement headcap for at least 2 days post implantation, permitting the pup to feed and to interact with the dam. We test both flat-tipped and minimally invasive custom tapered fibers to collect calcium and dLight fluorescence traces from the DMS in response to naturalistic stimuli in acute and chronic paradigms. Our protocol highlights the need for accessing neural substrates during early development and establishes an approach to probe dopamine dynamics in early postnatal mice.

Disclosures: M. Janecek: None. **R.T. Peixoto:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.02

Topic: A.07. Developmental Disorders

Support: CAUT22AFP011
TL1TR003019
Mindworks Charitable Lead Trust

Title: Early life antibiotic exposure alters postnatal neurogenesis in 16p11.2 neurodevelopmental disorder mice

Authors: *C. R. MCDERMOTT[1], A. MIRMAJLESI[2], X. ZHANG[3], X. ZHOU[1], J. H. MILLONIG[3], M. J. BLASER[3], E. M. DICICCO-BLOOM[1];
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Abstract: Neurodevelopmental disorders (NDDs) affect the lives of >17% of children and caregivers in the United States and result from the combination of genetic risk variants and exposure to environmental factors. The clinical heterogeneity of NDDs, even among those bearing the same genetic risk, suggests that environmental factors may act on certain genetic susceptibilities, making a Gene x Environment model warranted. A recently identified environmental factor that appears to be linked to increased NDD risk is exposure to cephalosporin antibiotics during the first 2 years of life. Extensive evidence indicates that antibiotic exposure decreases the diversity of gut bacteria (antibiotic-induced dysbiosis) which may consequently alter adult brain structure and behavior. However, little is known about its effects on developmental neurogenesis, which has been implicated in human genetic and animal model studies as a point of convergence in NDD pathogenesis. Our studies use a novel Gene x Environment model to determine how cephalosporin exposure alters postnatal neurogenesis in the genetically vulnerable 16p11.2 microdeletion (16pDel) mouse. This mouse models one of the

most frequently observed CNVs that accounts for ~1% of ASD and also results in other NDD phenotypes. To model early human life antibiotic exposure, wildtype (WT) and 16pDel littermates were exposed to water (control) or the cephalosporin, cefdinir, from postnatal (P) days 5-9 and sacrificed on P13 for analyses. Our preliminary studies examined the cerebellum and hippocampus, two regions that undergo postnatal neurogenesis throughout the first year of human life and are implicated in NDDs. Using immunoblot analysis, we found sex- and genotype-dependent effects of cefdinir exposure on cerebellar neuronal differentiation and hippocampal neurogenesis. Cefdinir-exposed WT mice had a 50% reduction in levels of hippocampal intermediate precursor marker, Tbr2, compared to controls. In genetically vulnerable 16pDel mice, cefdinir-exposed males had decreased hippocampal Cyclin E (mitosis marker) and increased doublecortin (differentiation marker) compared to controls, while cefdinir-exposed females had reduced levels of cerebellar doublecortin compared to controls. Thus, cefdinir effects were dependent on sex and genotype. These results collectively suggest that early life exposure to cephalosporin antibiotics alters postnatal neurogenesis in the genetically vulnerable 16pDel mouse. Ongoing work will assess effects on microbial community composition and function, neurodevelopmental gene expression, and behavioral outcomes related to NDDs.

Disclosures: C.R. McDermott: None. **X. Zhang:** None. **X. Zhou:** None. **J.H. Millonig:** None. **M.J. Blaser:** None. **E.M. DiCicco-Bloom:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.03

Topic: A.07. Developmental Disorders

Support: SFARI Award #: 385188
NICHD; P50 HD103573

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

Authors: *J. S. LORD, S. M. GAY, K. M. SMITH, K. M. HARPER, V. D. NIKOLOVA, S. S. MOY, G. H. DIERING;
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Abstract: Sleep is a life-long essential behavior that supports brain development and maturation in early life. Comorbid sleep pathologies occur in over 80% of individuals diagnosed with Autism Spectrum Disorders (ASD), including young children; the lasting consequences of that sleep loss are unknown. Shank3 is a high-confidence ASD risk gene, and heterozygous mutation

of Shank3 is known to cause Phelan McDermid Syndrome, a severe developmental disorder characterized by intellectual disability, autism, and disrupted sleep. In our study, a clinical model mouse bearing C-terminal truncation (ΔC) of Shank3 (Shank3$^{+/\Delta C}$) differed minimally from wild type littermates in amount and quality of sleep. We suspected that normal baseline sleep might mask an underlying vulnerability to the effects of developmental sleep loss in Shank3$^{+/\Delta C}$ mice. Therefore, we tested the lasting effects of early-life sleep disruption (ELSD) on Shank3$^{+/\Delta C}$ mice and wild type littermates through mild mechanical perturbation from postnatal days 14 - 21. We also subjected a group of post-adolescent mice to sleep disruption at 8 weeks old (PASD). Compared to undisturbed controls, adult male Shank3$^{+/\Delta C}$ exposed to ELSD but not PASD demonstrated a marked deficit in sociability in the 3-chambered social test, reduced open field activity, and impaired pre-pulse inhibition. Adult female Shank3$^{+/\Delta C}$ exposed to ELSD or PASD showed a reduction in anxiety-like behavior in the elevated plus maze. These results establish a novel gene x sex x environment interaction model of ASD susceptibility and support early life sleep disruption as a significant vulnerability in the development of ASD.

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

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.04

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01MH123661

Title: Sex-biased impacts of 16p11.2 hemideletion on the striatal proteome in mice

Authors: *M. J. MERFELD[1], K. SCHROEDER[2], E. WOOD[2], G. R. ROJAS[2], L. S. CURRY-POCHY[2], L. PARKER[3], N. M. GRISSOM[2];
[1]Univ. of Minnesota Grad. Program In Neurosci., Minneapolis, MN; [2]Dept. of Psychology, [3]Dept. of Biochemistry, Mol. Biol. and Biophysics, Univ. of Minnesota, Minneapolis, MN

Abstract: Many neurodevelopmental disorders, including autism spectrum disorder (ASD), intellectual and developmental disability (IDD), and attention deficit hyperactivity disorder (ADHD) are highly male-biased in their diagnosis. The 16p11.2 hemideletion is a copy number variation (CNV) that increases risk of diagnosis of these neurodevelopmental disorders. The risk of neuropsychiatric diagnoses is elevated in males relative to females with this CNV in human studies. To understand the molecular pathophysiology and sex-biased impacts of this genetic deletion, we used mice carrying a hemideletion of chromosome 7qF3 that corresponds to the 16p11.2 CNV region observed in humans. Previous work identifies sex-biased impacts in striatal

function in this mouse model, in particular, protein expression of the mapk3/ERK1 gene located in the CNV region. To explore global protein expression in the striatum, which may be altered by 16p11.2 hemideletion, we examined the striatal proteomes of male and female 16p11.2 hemideletion (n=3 per sex) and wild-type (n=2 per sex) mice. Scaffold Q+ was used to quantitate isobaric tagging for relative and absolute quantification (iTRAQ) peptide and protein identification. There were 5969 discernible proteins found in the striatum, only 9 of which are known to be affected by 16p11.2 hemideletion. Fitting with previous results from our lab (Grissom et al 2018), we found a downregulation of mapk3 protein in both sexes with the hemideletion, compared to wild-type controls. Preliminary analysis indicates that more proteins were found to be significantly different between male wild-type mice versus male 16p11.2 hemideletion mice compared to females across genotypes, supporting the hypothesis that males are differentially impacted by this genetic deletion. In addition, the vast majority of the proteins found to be differentially expressed have genetic loci outside of the 16p11.2 region. Analysis is ongoing at this time, with parallel reaction monitoring-mass spectrometry (PRM-MS) planned for targeted confirmation of these results.

Disclosures: M.J. Merfeld: None. **K. Schroeder:** None. **E. Wood:** None. **G.R. Rojas:** None. **L.S. Curry-Pochy:** None. **L. Parker:** None. **N.M. Grissom:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.05

Topic: A.07. Developmental Disorders

Support: R01-MH118563-01

Title: Social ranking is disrupted in Rett syndrome mice: exploring mPFC neuronal ensembles reflecting social hierarchy

Authors: *C. ACEVEDO-TRIANA, L. POZZO-MILLER;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Alterations in social behaviors are considered a key feature in several developmental neuropsychiatric disorders, such as autism and schizophrenia. The medial prefrontal cortex (mPFC) is involved in cognitive functions, behavioral regulation and flexibility, as well as social behaviors, and is dysfunctional in neurodevelopmental disorders due to its protracted postnatal development. Recent work in our lab showed that direct ventral hippocampal (vHIP) inputs to the mPFC regulate social memory, and are dysfunctional in *Mecp2* knockout (KO) mice, an experimental model for the syndromic autism Rett syndrome. However, how alterations in this vHIP-mPFC pathway affect the formation of social ranks, which require intact social memory, is

unknown. Here, we first confirmed that *Mecp2* KO mice don't have deficits in odor habituation/dishabituation, showing the expected preference for social stimuli observed in WT mice that is necessary to perform social tasks. Next, we used the social dominance "tube" test to identify the social ranking of 3-4 previously isolated mice. The hierarchy of social ranking of either WT or *Mecp2* KO mice housed together was established through 6 daily pairwise competitions using a round-robin format; winners were those mice that either pushed the opponent or waited for its retreat and exit the tube through the opponent's side; the mice with more wins were considered to be in the highest social rank. WT mice showed a stable hierarchy over 6 consecutive days, while *Mecp2* KO mice failed to show a stable ranking of winners. Behavioral ethograms built semi-automatically revealed that WT and *Mecp2* KO mice showed similar behaviors of dominance when they won the test (pushing forward, resistance), but the *Mecp2* KO winners showed submissive behaviors (slow retreat or remaining still inside the tube) more frequently than WT mice. Finally, WT mice with GCaMP8 expression in neurons of the prelimbic (PL) subregion of the mPFC were unilaterally implanted with a GRIN lens over the mPFC to image Ca2+ signals (as surrogates of neuronal spiking) with head-mounted miniscopes during the "warm-spot" test, an unrestricted social dominance test that allows the use of wired head-mount miniscopes. Different mPFC neuronal ensembles were specifically activated during the "warm spot" test as opposed to during unrestricted interactions with a WT mouse, indicating that this approach could be useful to compare and contrast with mPFC social ensembles in *Mecp2* mutant mice. We expect that identifying mPFC neuronal ensembles encoding social hierarchy will allow a deeper understanding of their contribution to altered sociability in Rett and other MeCP2 associated disorders.

Disclosures: C. Acevedo-Triana: None. **L. Pozzo-Miller:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.06

Topic: A.07. Developmental Disorders

Support: NIH/NICHD, R01 HD099162

Title: The role of the E3 ubiquitin ligase UBE3B in autism spectrum disorder

Authors: *S. VASHISTH, I. TUNCAY, K. KAUR, M. CHAHROUR;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired communication, abnormal social behaviors, and restricted and repetitive behaviors. Pathogenic mutations in *UBE3B* result in neurodevelopmental disease including intellectual

disability, complete lack of speech, and ASD. *UBE3B* encodes an E3 ubiquitin ligase, which functions in the ubiquitin-proteasome system (UPS) to tag substrate proteins with ubiquitin for degradation by the proteasome. The UPS is known to regulate several signaling pathways critical in neurodevelopment, including neurogenesis and synapse formation and function, and mutations in various UPS genes have been identified in ASD. To investigate the function of UBE3B and its contribution to ASD, we generated a brain-specific nestin-Cre conditional knockout (cKO) mouse model and evaluated ASD-like behaviors in neonatal and juvenile cKO mice. We found that cKO pups emit significantly fewer, shorter, and quieter ultrasonic vocalizations (USVs) when separated from their mothers compared to control pups, indicating a neurodevelopmental abnormality. In a direct social interaction task, cKO juveniles spent less time interacting with unfamiliar, age- and sex-matched conspecifics. In addition, we found that cKO mice had a slower mean latency to the target location than control mice in the Morris Water Maze task, suggesting that loss of UBE3B leads to deficits in learning and memory. To identify potential neuronal UBE3B substrates, we used stable isotope labeling by amino acids in cell culture (SILAC) of neural stem cells from wild type (WT) and constitutive *Ube3b* knockout (KO) mice. Selected candidates exhibited increased protein level and decreased ubiquitination in KO compared to WT cells. Gene ontology analysis indicated that the majority of identified candidate substrates are involved in synaptic function. Our findings suggest that brain-specific *Ube3b* deletion leads to ASD-like behaviors and that UBE3B may be involved in the synaptic function underlying these behaviors. Ongoing and future studies will further investigate the neuronal substrates of UBE3B and how loss of UBE3B alters synaptic transmission.

Disclosures: S. Vashisth: None. **I. Tuncay:** None. **K. Kaur:** None. **M. Chahrour:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.07

Topic: A.07. Developmental Disorders

Support: Dutch Research Council (NWO, ZonMw) Talent Programme Vidi
Dutch Research Council (NWO) XS Grant

Title: Effects of TSC1 haploinsufficiency in the developing murine cerebellum

Authors: *I. SERRA[1], A. STRAVS[2], C. OSORIO[1], C. TUDORACHE[2], A. BADURA[1];
[1]Erasmus Med. Ctr., Rotterdam, Netherlands; [2]Leiden Univ., Leiden, Netherlands

Abstract: Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder of heterogenous phenotypes, characterized by the presence of social deficits, and inflexible and repetitive behaviours. Despite its unknown etiology, several genetic variants have been

associated with increased ASD risk. Mutations in tuberous sclerosis complex 1 (*Tsc1*), a tumour suppressor gene that inhibits the mechanistic target of rapamycin (mTOR) pathway, leads to a disorder of the same name in which up to half of patients present with ASD. Amongst the different brain areas proposed to play a role in the development of ASD, the cerebellum is commonly reported to be altered, with atypical cell morphology, structure and altered cerebello-cortical connectivity found in people with this disorder. Furthermore, cerebellar-specific deletion of *Tsc1* in mice is sufficient to induce an ASD-like phenotype. Given, that the mTOR pathway is crucial for proper cell replication and migration, this suggests that dysregulation of this pathway, particularly during critical phases of cerebellar development, could contribute to the establishment of ASD. Here, we used a mouse model of Tsc1 to investigate gene and protein expression during embryonic and early postnatal periods of cerebellar development. We found that, at E18, mRNA levels of cerebellar interneuron and glial markers were decreased. This was accompanied by a downregulation of mTOR-related genes. Further, while at postnatal day 1, mRNA levels were comparable between mutants and controls, at P7, interneuron markers were increased in Tsc1 mutants. Additionally, differential gene correlation analysis revealed dynamic changes in gene pairs across development, with an overall loss of correlation between mTOR- and cerebellar-related genes in Tsc1 mutants compared to controls. These dynamic changes were also found across behavioural tasks, where animals were repeatedly tested both at juvenile and adult timepoints. Our findings indicate that E18 is a crucial timepoint in cerebellar development particularly susceptible to mTOR pathway dysregulation. This leads to changes in the regulation of genes contributing to the excitatory/inhibitory balance, a mechanism proposed to be involved in the etiology of ASD, which later likely impacts the behavioural presentation of juvenile and adult mice.

Disclosures: I. Serra: None. **A. Stravs:** None. **C. Osorio:** None. **C. Tudorache:** None. **A. Badura:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.08

Topic: A.07. Developmental Disorders

Support: SFARI Bridge to Independence Award, to X.T.

Title: Restoration of disease risk gene expression to treat neurodevelopmental disorders

Authors: *X. TANG;
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Abstract: Neurodevelopmental disorders (NDD) affect one in six children in the US. Upwards of 90% of risk for NDD is genetically conferred by a large number of disease risk gene variants. Contrary to the common view that the NDD-related abnormalities caused by genetic mutations are cemented after brain development, it was demonstrated that replacing mutant genes experimentally in adult mice can largely rescue disease-related phenotypes, providing the basis for the ongoing effort to develop a gene therapy approach to treat NDD in patients. However, an intrinsic challenge to this approach is the limited applicability of expensive, personalized precision gene therapies for the majority of NDD patients presenting a broad range of mutations in one or multiple NDD risk genes. Therefore, it is critical to determine the common NDD disease mechanisms for developing broadly applicable therapeutic strategies.
We present recent work aiming to identify common molecular-level abnormalities induced by various NDD risk gene mutations (such as a reduction in KCC2 expression), and to develop pharmacological approaches (such as inhibition of the FLT3 kinase pathway) that rectify the perturbations in the expression level of disease risk genes to relieve NDD symptoms regardless of the complexity or uncertainty of the causes.

Disclosures: X. Tang: None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.09

Topic: A.07. Developmental Disorders

Support: Farrehi Family Foundation Grant
NIH Grant T32-NS076401
NIH Grant T32HD079342
NIH Grant NS089896
NIH Grant NS116008

Title: Ectopic expression of germline genes with loss of histone demethylase KDM5C in X-linked intellectual disability

Authors: *K. M. BONEFAS[1,2], S. IWASE[1];
[1]Human Genet., [2]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Chromatin regulators are an important component of the epigenetic environment that can influence transcription to ultimately shape cellular identity. Currently, it is unclear why mutations in numerous chromatin regulators cause neurodevelopmental disorders (NDDs). For example, lysine demethylase 5c (KDM5C) erases the histone 3 lysine 4 trimethylation (H3K4me3) and loss-of-function mutations in *KDM5C* result in intellectual disability,

heightened aggression, and comorbidity with Autism Spectrum Disorder. Surprisingly, we found the *Kdm5c*-KO mouse amygdala and hippocampus — two brain regions important for these behaviors — aberrantly express many germline genes that have no known function in the brain. We found this apparent soma-to-germline transition occurs during early *Kdm5c*-KO embryogenesis and parallels germ cell development. This failure of *Kdm5c*-KO embryos to demarcate somatic and germline identity results in aberrant expression of germline genes across multiple tissues. Ectopic transcription of germline genes in the brain suggests *Kdm5c*-KO neuronal function may be impaired due to a failure to faithfully establish neuronal identity. Our work provides mechanistic insight into how KDM5C helps delineate the somatic and germline transcriptomes. This work also offers early insight into a novel mechanism by which chromatin regulators are essential for neurodevelopment and behavior.

Disclosures: K.M. Bonefas: None. **S. Iwase:** None.

Poster

P049. Autism: Insights from Syndromic Autism Studies

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P049.10

Topic: A.07. Developmental Disorders

Support: 5R01NS104428-03

Title: Sodium-dependent citrate transporter (SLC13A5) mutant mice suggest that dysfunction is not limited to citrate transport deficiency

Authors: *L.-J. CHEW[1,2], K. A. DE LEON[3], J. F. CARR[1], S. L. HELFAND[1], J. S. LIU[1,4,2]; [1]Dept of Mol Biol, Cell Biol and Biochem., [2]Ctr. for Translational Neurosci., [3]Dept of Neurosci., Brown Univ., Providence, RI; [4]Dept of Neurol., Rhode Island Hosp. and Warren Alpert Med. Sch. of Brown Univ., Providence, RI

Abstract: Mutations in the SLC13A5 gene, which encodes a plasma membrane citrate transporter, result in early infantile epileptic encephalopathy, a neonatal neurological disorder characterized by multi-focal seizures. Human genetics has identified commonly occurring missense and deletion mutations that abolish citrate transport, but it is not known how distinct mutations affect disease severity. We have chosen to address this by characterizing an array of SLC13A5 mutant mice: i) ablation of endogenous Slc13a5 gene (knockout), ii) the most common patient mutation, the G222R point mutation (equivalent to the human mutation G219R), and iii) the second most common patient mutation, the T230M (equivalent to the human mutation T227M). Our preliminary data indicates that at 3 months of age, the homozygous Slc13a5 knockout mouse, which shows abnormal neurotransmitter levels, and heterozygous T230M/+ mutant, both demonstrate mild epileptiform electroencephalogram (EEG) profiles.

Heterozygous and homozygous G222R point mutation mice have more severe epileptiform activity, but severe status epilepticus is observed only in heterozygous G222R/+ mice, suggesting a toxicity distinct from citrate transport deficiency. These changes in EEG spike activity are accompanied by corresponding tissue damage that is most severe in heterozygous G222R/+ brains, including reduced parvalbumin+ interneurons, MAP2, and increased astroglial hypertrophy in cerebral cortex and hippocampus. There is a loss of oligodendrocytes and increased astroglial reactivity in subcortical white matter, indicating widespread pathology. In HEK293T cells which lack endogenous SLC13A5, recombinant human SLC13a5 and T227M are localized at the plasma membrane. G219R mutant protein impairs cell growth, fails to colocalize in the plasma membrane, and appears perinuclear, colocalizing with mitochondrial markers Mitotracker Red, cytochrome C and Tomm20. Co-expression of the G219R mutant together with its wild-type counterpart prevents membrane localization of the wild-type protein, an event reversed by increasing concentrations of wild-type construct. Metabolic studies in HEK293T cells transfected with G219R revealed an increased rate of oxygen consumption and basal respiration, which suggests mitochondrial dysfunction. These results indicate that neuronal hyperactivity is differentially regulated by distinct SLC13A5 mutations. Loss of citrate transport function underlies epileptiform activity, likely due to altered neurotransmitter levels. However, specific missense mutations may acquire dominant gain-of-function effects which exacerbate neuronal hyperexcitability.

Disclosures: L. Chew: None. **K.A. De Leon:** None. **J.F. Carr:** None. **S.L. Helfand:** None. **J.S. Liu:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.01

Topic: A.09. Adolescent Development

Support: Wayne State Department of Psychiatry and Behavioral Neurosciences
National Institute of Mental Health

Title: Lead exposure risk correlates with anxiety symptoms in urban youth

Authors: *J. LOSIOWSKI, B. BORG, J. EVANSKI, H. MARUSAK;
Wayne State Univ., Detroit, MI

Abstract: Lead is a known neurotoxic metal, and lead-exposed children experience disrupted cognitive and behavioral development (i.e., lower IQ, poorer academic achievement, hyperactivity, antisocial behavior). While lead affects both urban and non-urban settings, children growing up in urban environments are at particularly high risk of lead exposure due to a

high concentration of automobile and industrial emissions, older homes, and water systems, where lead is commonly found. Though the detrimental effects of lead exposure on cognitive and behavioral health have been well documented, less is known about the impacts on *mental health*. The present study examined the effects of lead exposure risk on anxiety in youth living in urban vs. non-urban settings. Sixty-five adolescents (37 female) aged 10-17 years (mean age=13.05 $\pm$ 2.20) completed an online survey about mental health and environmental exposures. We split the sample into two groups: urban (n=19), defined as living in the city of Detroit, and non-urban (n=46), defined as living >50 miles of Detroit. Youth self-reported on their anxiety symptoms using the Screen for Child Anxiety Related Emotional Disorders questionnaire. Lead exposure risk was estimated using the zip code of the youth's current residential address. In particular, we used data from Data Driven Detroit to estimate the percent of children who tested positive for elevated blood lead levels in each participant's zip code from 2017-2019. Linear regression was used to test the impact of lead exposure risk on anxiety across the entire sample and in urban and non-urban subgroups, separately. Self-reported gender was used as a covariate in all analyses. Overall, lead exposure risk did not significantly predict anxiety across the entire sample. However, when considering urban and non-urban environments separately, lead exposure risk was a significant positive predictor of anxiety symptoms in urban (but not non-urban) youth. Our results revealed that a higher risk for lead exposure is associated with higher anxiety symptoms in youth living in urban (but not non-urban) settings. These data fit with a small but growing number of studies documenting detrimental effects of childhood lead exposure on lifelong mental health. Taken together, these data indicate that environmental exposures, such as lead exposure, can operate as unseen drivers of poor mental health, particularly in urban settings. These data also highlight the role of public policies to prevent lead exposure in vulnerable populations, including children and adolescents.

Disclosures: J. Losiowski: None. **B. Borg:** None. **J. Evanski:** None. **H. Marusak:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.02

Topic: A.09. Adolescent Development

Support: FAPESP 2019/19709-6
FAPESP 2016/14750-0
CNPq – #301899/2019-3
CAPES finance code 001
AFIP

Title: Beyond age in adolescence cognition: a latent factor of puberty predicts development of executive functions ability in early adolescence.

Authors: S. POMPEIA, ***I. A. SEGURA**;
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Abstract: Executive Functions (EFs) develop during adolescence, an age when it is possible to observe the differentiation of EFs into three correlated but separable domains according to the Unity and Diversity Model of EFs. The improvement in EFs is believed to be due to profound structural and functional brain changes which can be partly due to experiences gained as adolescents age and/or to pubertal factors, which vary greatly among individuals in terms of onset and progression. However, pubertal effects on the development of EFs are often overlooked or, when they are controlled for, this is usually done using a single pubertal milestone or scores in a single scale. This is not ideal because puberty involves many physiological changes that are not captured by any single marker. The aim of the present study was to investigate the association of a latent variable of puberty with EFs factors from the EF Unity and Diversity Model in early adolescence, when puberty occurs. A total of 332 adolescents (188 girls) were tested cross-sectionally. The pubertal latent factor was built using a unifactorial Confirmatory Factor Analysis (CFA) solution with five indicators of pubertal status: 1) the Pubertal Developmental Scale (filled in by both parents and adolescents); 2) self-assessed Tanner staging (one measure of gonadal and one of adrenal development); and 3) salivary level of the dehydroepiandrosterone sulfate (testosterone did not load well on the model). Next, Structural Equation Modeling (SEM) was used to regress the puberty factor, controlled for age (in months), onto the three correlated factors of EFs, each composed by scores in two tasks: Updating (2-Back and Number Memory tests), Inhibition (Stroop Victoria and Stroop Happy-Sad) and Switching (Color Shape and Category Switch). Measurement CFA models were stablish for both EFs and puberty. The intercorrelated three-factor EF model was replicated and both CFA solutions presented good fit to the data with moderate to high factor loadings. The subsequent SEM model also retrieved adequate fit indices and all paths were statistically significant. In accordance with what is found with age, a higher pubertal latent trait predicted better EF in all the three domains, even after controlling for age. We conclude that puberty can be used as a construct to understand cognitive development because the puberty latent factor significantly predicted EFs abilities (controlled for age), reinforcing the importance of considering multiple pubertal measures when assessing cognition during adolescence.

Disclosures: S. Pompeia: None. **I.A. Segura:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.03

Topic: A.09. Adolescent Development

Title: The potential for GENUS as an early intervention to prevent negative impact of early life adversity on later-life prosocial behavior.

Authors: *T. BEHRING[1], O. AGU[1], E. SHER[1], G. HASSAN[2], A. MANGANARO[1], R. LUDWIG[1], M. M. MYERS[1], M. G. WELCH[1], D. DUMITRIU[1];
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Abstract: Prosocial behavior is imperative to functioning in society. A lack of prosocial behavior is hallmark of many psychopathologies, including autism spectrum disorders (ASD), schizophrenia, and antisocial personality disorder. The prevalence of antisocial personality disorder is 1-4% of the population and has strong associations to early life stress (ELS). The mother-infant relationship is theorized to be the evolutionary antecedent to prosocial behavior and disruption of this relationship is a form of ELS associated with antisocial personality disorder and decreased prosocial behavior. In rats, the limited bedding and nesting (LBN) model, a form of ELS, is an effective disruptor of normal dam-pup interactions with long-lasting biobehavioral consequences. However, rodent models of ELS have not been used to study long-lasting prosocial behavioral deficits. The trapped-rat paradigm is a reliable and ethologically valid tool for testing prosocial behavior in rats. In prior work, we found cortical gamma power (~40 Hz), which is important for developing circuit plasticity and emotion regulation, to be decreased by ELS in rat pup frontal cortex. Gamma ENtrainment Using Sensory Stimuli (GENUS) has potential for rescuing deficits in cortical gamma power and has been used to treat cognitive and emotional deficits in other psychopathologies, such as Alzheimer's disease. Here, we present data on later-life prosocial behavioral deficits in LBN rats. From the ages of P2 to P9 LBN rats were reared in limited bedding conditions (100 mL corncob) and were tested on the trapped-rat paradigm during adulthood. LBN rats showed a decrease in prosocial behavior compared to their control counterparts. We further present preliminary findings that early life GENUS, using a flickering light, can entrain cortical gamma power during early development (P17-23). Our future studies will focus on replicating these results and testing the hypothesis that GENUS during early life will rescue later life prosocial behavioral deficits seen in our ELS rat model.

Disclosures: T. Behring: None. **O. Agu:** None. **E. Sher:** None. **G. Hassan:** None. **A. Manganaro:** None. **R. Ludwig:** None. **M.M. Myers:** None. **M.G. Welch:** None. **D. Dumitriu:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.04

Topic: A.09. Adolescent Development

Support: FAPESP #2017/06100-8

Title: Brain development during adolescence and the impact on psychiatric disorders and drug abuse: a scoping review

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Abstract: Adolescence is a period of transformations in which new experiences are fundamental for the transition between childhood and adulthood. During this period, there is a drastic change in behavior, characterized by lack of impulse control and increased emotional response. Although this behavioral change can be essential for human development, it is also related to problems such as alcohol and drug abuse, as well as psychiatric disorders. Our objective is: a. to review scientific articles on human brain development that permeate the reckless behavior of adolescents; b. to understand the motivations underlying those behaviors; c. to disseminate the information found, to assist in preventive actions to the problems faced by adolescents.
Methods: A scoping review was carried out through the synthesis of articles published on the theme "Adolescent brain development", in the Medline database (PubMed) for the last 15 years. The filters "reviews" and "study in humans" were selected. Finally, a characterization was made in relation to the year of publication, country of origin and impact factor of the published journal.
Results: The analysis and synthesis of the 46 selected articles (out of 1,913) demonstrated that the period of adolescence is marked by a process of expressive changes in brain maturation. These occur asynchronously on different regions: primarily in areas of lower complexity (e.g. limbic subcortical systems) and later in more complex areas (e.g. cortical systems). Thus, in the adolescent brain, the limbic reward systems are already fully developed, while the cortical regulatory systems, still immature, are insufficient to perform adequate control, impairing the ability of adolescents to exert control over emotions, modify impulses and postpone gratification. Consequently, adolescence is characterized by an increase in risk behaviors and search for immediate rewards (e.g., drug abuse). Also, during this period, there is an increase in the incidence of psychiatric disorders, since the subcortical regions, which are less regulated during the maturation process (e.g., nucleus accumbens, amygdala), are the same described as altered in the pathology of the most common mental disorders (e.g., anxiety and depression). **Conclusion:** During adolescence, the active process of cerebral maturation characterizes a late plasticity, but the disparity between reward and control systems makes them more likely to suffer from various risks. Therefore, understanding the motivations behind the actions of this group is fundamental to ensure that environmental influences reach adolescents in a positive way, when developing strategies in public policies.

Disclosures: S. Chiavegatto: None. **L.R. Poian:** None. **L.D. Lamarca:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.05

Topic: A.09. Adolescent Development

Support: NIH Grant R01-MH086507
NIH Grant R01-MH123147
NIH Grant R01-MH127850

Title: Disruption of prefrontal GABAergic maturation during adolescence by early life adversity

Authors: *E. ARTUR DE LA VILLARMOIS, E. FLORES-BARRERA, K. Y. TSENG; Dept. of Anat. & Cell Biology, Univ. of Illinois at Chicago, CHICAGO, IL

Abstract: Early life experiences have been known to impact the development and maturation of corticolimbic connectivity and its regulation of cognitive and affective behaviors. For instance, individuals with a history of early life trauma are often at higher risk for anxiety-like disorders in adolescence, which are thought to result from a dysregulation of affective and cognitive processes, and the maturation of the prefrontal cortex (PFC) and associated neural circuits. It is therefore conceivable that early life events contribute to shape the developmental trajectory of PFC maturation during adolescence. To test this idea, we implemented an early life adversity paradigm (i.e., maternal separation, MS) and assessed to what extent PFC maturation of synaptic activity from postnatal day (P) 30 to adulthood becomes disrupted in both male and female rats. Data revealed that the normal developmental gain of PFC GABAergic synaptic activity was not observed in animals exposed to MS, while AMPA-mediated transmission was not affected. As a result of the selective GABAergic disruption in MS animals, the excitatory-inhibitory (E-I) ratio that normally becomes balanced by P50 remains unbalanced in adulthood (e.g., >1.0), resembling the level of E-I synaptic activity typically found in the immature PFC of P30-40 rats. Furthermore, transient chemogenetic inhibition of PFC GABA interneurons during this early adolescent period was sufficient to elicit a similar E-I imbalance that endures through adulthood. Collectively, our findings indicate that PFC inhibitory synapses are preferentially susceptible to early life adversity, likely through disruption of an activity dependent mechanism that is needed to enable the GABAergic maturation during adolescence. In turn, a disinhibited PFC state could increase the risk for psychiatric disorders during adolescence with abnormal affective and cognitive responses.

Disclosures: E. Artur De La Villarmois: None. **E. Flores-Barrera:** None. **K.Y. Tseng:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.06

Topic: A.09. Adolescent Development

Support: UWRF Undergraduate Stipends and expenses grant

Title: The effect of adolescent isotretinoin exposure on the hippocampus, depressive-like behavior, and recognition memory

Authors: *C. T. PETERSON[1], K. P. G. LAROCCO[1], H. R. KOST[1], P. J. LISOWSKI[2], D. G. EHLINGER[1];
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Abstract: 13-cis-retinoic acid (Isotretinoin) is a commonly prescribed medication for the treatment of persistent cystic acne but has also been shown to cause depressive behaviors and suicidal thoughts in patients. The hippocampus is a brain area involved in regulation of the stress response as well as cognitive functions such as novel memory formation, and alterations to this area may be important to the development of symptoms following adolescent isotretinoin exposure. In the present study, we assessed the effects of adolescent isotretinoin exposure on stress-coping behavior and novel object recognition, followed by measurement of hippocampal dopamine D2 receptor expression and dendritic spine density. From approximately postnatal day (P)35 through P49, C57BL/6J mice were injected with 1 mg/kg of isotretinoin intraperitoneally for 10 total injection days. From approximately P50 through P52, mice underwent novel object recognition testing, followed by a 6-minute forced swim test on approximately P53. Brains were extracted for western blotting of dopamine D2 receptor density in the hippocampus and striatum, and Golgi-Cox tissue staining for analysis of dendritic spine density. To date, our results indicate an increase in immobility in the forced swim test, but no effect on novel object recognition, in isotretinoin exposed mice compared to controls. These results suggest that adolescent isotretinoin exposure is associated with depressive-like behavior in mice but does not influence hippocampal dependent memory formation. Ongoing research aims to correlate hippocampal dopamine D2 receptor expression and dendritic spine density with our behavioral results.

Disclosures: C.T. Peterson: None. **K.P.G. LaRocco:** None. **H.R. Kost:** None. **P.J. Lisowski:** None. **D.G. Ehlinger:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.07

Topic: A.09. Adolescent Development

Support: UWRF Undergraduate Stipends and Expenses Grant
UWRF Faculty Research Grant

Title: The effect of adolescent social isolation on nicotine conditioned place preference and stress-coping behavior.

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Abstract: Adolescence is a sensitive period in brain development that is marked by increased susceptibility to chronic stress and the reinforcing effects of drugs of abuse, which may enhance vulnerability to neuropsychiatric conditions such as depression and substance use disorders. In the present study, we used an animal model to examine the effect of adolescent social isolation stress on coping behavior and nicotine reward. During the adolescent period from postnatal day (P)35-P49, male and female C57BL/6J mice were exposed to either social isolation (SI) stress or standard rearing (SR) conditions, as well as nicotine exposure (0.35mg/kg) four times between P35-P49 during a nicotine conditioned place preference (CPP) procedure. On approximately P50, stress-coping behavior was examined following a 6-minute forced-swim test (FST). Our behavioral results show that both male and female SI mice more rapidly develop nicotine CPP compared to SR mice, and that female SI mice exhibit increased levels of immobility in the FST. These results suggest that adolescent social isolation stress enhances the rewarding effects of nicotine and negatively impacts stress-coping behavior. Ongoing research efforts are examining structural (dendrite remodeling and spine density) and functional (c-fos expression) differences in the brains of SI versus SR mice using Golgi-Cox tissue staining of the dopaminergic reward pathway and immunohistochemistry of the ascending serotonergic system. Collectively, these analyses will help determine neurological correlates of adolescent susceptibility to the negative effects of chronic social isolation stress and inform our understanding of adolescent brain development and vulnerability.

Disclosures: A.C. Kirckof: None. **D.G. Ehlinger:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.08

Topic: A.09. Adolescent Development

Support: Swiss National Science Foundation grant (310030_185192 to Dr Benjamin Boutrel)

Swiss Government Excellence Scholarship to Dr. Kshitij Jadhav
Doc mobility fellowship from the SNSF to Dr. Kshitij Jadhav

Title: Rescuing anterior insula cortex neuronal hypoexcitability attenuates compulsive behavior in juvenile rats

Authors: *K. JADHAV[1], A. BERNHEIM[1], L. AESCHLIMANN[1], G. KIRSCHMANN[2], I. DECOSTERD[2], A. F. HOFFMAN[3], C. R. LUPICA[4], B. BOUTREL[5];
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Abstract: Development of self-regulatory competences during adolescence is partially dependent on normative brain maturation. Here we report that juvenile rats as compared to adult rats exhibit impulsive and compulsive-like behavioral traits, the latter being associated with lower expression of zif268 mRNA levels in the anterior insula cortex but not in the prelimbic cortex, possibly reflecting immature integration of interoceptive cues in rational decision-making. This is further substantiated with hypoexcitability of layer-V pyramidal neurons of the anterior insula cortex using slice electrophysiology, concomitant with lower excitatory glutamatergic inputs and ultimately lower functional recruitment of the anterior insula in juvenile rats as compared to adult rats. Importantly these electrophysiological differences between adolescent and adult rats were not observed in the layer-V pyramidal neurons of the prelimbic cortex. Chemogenetic rescue of the anterior insula cortex pyramidal neurons attenuated the compulsive trait suggesting that immaturity of the anterior insula cortex to integrate sensory and cognitive information in youngsters may contribute to inflexible behaviors in specific conditions of reward availability.

Disclosures: K. Jadhav: None. **A. Bernheim:** None. **L. Aeschlimann:** None. **G. Kirschmann:** None. **I. Decosterd:** None. **A.F. Hoffman:** None. **C.R. Lupica:** None. **B. Boutrel:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.09

Topic: A.09. Adolescent Development

Support: U01DA041022
U01DA041028
U01DA041048
U01DA041089

U01DA041106
U01DA041117
U01DA041120

Title: Dietary pattern is associated with mental health in early adolescence: findings from a large population-based study

Authors: *C. SHETH, R. HUBER, E. MCGLADE, P. RENSHAW, D. YURGELUN-TODD; Univ. of Utah, Salt Lake City, UT

Abstract: Robust evidence in nutritional psychiatry has highlighted the relationship between healthy dietary patterns and positive mental health throughout the life span. In late childhood and adolescence, eating behavior and social environment of eating change considerably as youth gain autonomy around food choices. This is also a time period wherein a number of mental health problems start emerging. The relationship between diet and mental health has been relatively under-investigated in children and adolescents. Studies have linked higher child behavior checklist (CBCL) scores with lower fruit and vegetable intake and with increased intake of processed foods. Putative mechanisms underlying this association include: 1) fruits/vegetables may provide the folate required for neurotransmitter synthesis critical for mood and emotion regulation 2) processed food has been associated with increased neuroinflammatory markers. However, most of these studies have been limited by small sample size as well as confounding contextualizing factors such as socioeconomic status. The Adolescent Brain Cognitive Development Study ^{SM} (ABCD Study®) a longitudinal, multi-site study has recruited a sociodemographically diverse cohort of more than 11,000 children with the goal of investigating a wide range of factors that influence developmental trajectories across adolescence. Here, we examine the cross-sectional association between dietary pattern and behavioral impairment in the ABCD sample at Year 1 follow-up. Dietary pattern was assessed with child nutrition questionnaire administered to the caregiver asking about frequency of consumption of 14 food categories in the past week. Higher scores indicate healthier dietary patterns. The CBCL was used to assess the mental health and behavior. Generalized additive mixed models, which modelled family nested within site as random effects, were used to examine the statistical relationship between dietary scores and CBCL scores. The following fixed effects covariates were used child's age, child's body mass index (BMI), waist circumference, sex, race, ethnicity, parental higher education, family income and marital status. Our results showed a significant association between dietary scores and CBCL scores such that higher scores on the nutrition questionnaire were associated with lower CBCL scores after controlling for covariates. These results suggest that a healthy diet (more fruits, vegetables and lower fast and processed food) may be associated with better mental health in early adolescence. Thus, modifying dietary behaviors may be a crucial strategy for maintaining optimal mental health in this age group.

Disclosures: C. Sheth: None. **R. Huber:** None. **E. McGlade:** None. **P. Renshaw:** None. **D. Yurgelun-Todd:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.10

Topic: A.09. Adolescent Development

Support: NIH Grant R01-MH-086507 to KYT

Title: Early adolescent inhibition of prefrontal GABAergic activity disrupts the maturation of prefrontal E-I balance in adulthood.

Authors: *E. FLORES-BARRERA, E. ARTUR DE LA VILLARMOIS, A. CABALLERO, K. Y. TSENG;
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Abstract: Understanding the processes that contribute to prefrontal cortex (PFC) maturation are crucial to reveal the neural mechanisms underlying the development and onset of major psychiatric disorders such as affective and substance use disorders, and schizophrenia. A hallmark of PFC maturation is the *re-calibration* of an excitatory-inhibitory (E-I) balance during adolescence. Here we asked the extent by which local GABAergic activity in the PFC during adolescence plays a role in the functional acquisition of an enhanced E-I control of PFC output in adulthood. Towards this goal, we implemented a chemogenetic approach to inhibit PFC GABA interneurons by using an inhibitory DREADD driven by an interneuron-specific DLX promoter. Using *ex-vivo* and *in vivo* electrophysiological measures, we found that transient inhibition of PFC GABAergic interneurons within the early adolescent window prevents the maturation of local GABA function and the normal inhibitory control of afferent drive without altering the level of AMPA-mediated synaptic transmission. As a result of the selective inhibitory disruption, the AMPA/GABA ratio in the PFC remains >1.0 as seen in P35-45 animals. Notably, this period of sensitivity to transient inhibition of PFC GABA interneurons was no longer apparent after P45. Collectively, these results indicate that an activity dependent mechanism contributes to the functional maturation of PFC GABAergic function during adolescence.

Disclosures: E. Flores-Barrera: None. **E. Artur de la Villarmois:** None. **A. Caballero:** None. **K.Y. Tseng:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.11

Topic: A.09. Adolescent Development

Support: A.Morgunova: This research was undertaken thanks in part to funding from the Canada First Research Excellence Fund, awarded to McGill University for the Healthy Brains, Healthy Lives initiative.
C.Flores: supported by the National Institute on Drug Abuse (R01DA037911), the Canadian Institutes for Health Research (MOP-74709; MOP-119543), and the Natural Science and Engineering Research Council of Canada (2982226)

Title: Mir-218 as a potential target to regulate prefrontal cortex development in adolescence

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Abstract: Adolescence is a period of increased vulnerability to psychiatric disorders, including major depressive disorder (MDD), and there is a need for markers to identify individuals at risk. Our studies on male mice found that miR-218 regulates expression of the guidance cue receptor DCC, which controls prefrontal cortex (PFC) maturation in adolescence, and functions as a switch between vulnerability and resilience to depressive-like traits in adult mice. miR-218 is downregulated in the adult PFC of individuals with MDD who died by suicide in comparison to psychiatrically-healthy sudden death controls. Furthermore our rodent findings show that microRNA levels in blood can match expression in the brain, and elevated "adult-like" miR-218 levels in adolescence predict risk to stress-induced behavioral abnormalities in adulthood. Our next objective is to assess the impact of altered miR-218 levels in adolescence via translational non-invasive manipulations, and to begin investigating the role of miR-218 in female rodents. In male mice, we administered anti-sense oligonucleotides (antagomiR-218 or a scrambled sequence) with intranasal spray and characterized behavioral traits. Mice that received intranasal antagomiR-218 in adolescence showed anxiolytic-like behaviors on elevated arms maze as adults ($p=0.033$, $n=20$), consistent with effects observed following adolescent antagomiR-218 intra-PFC microinfusions. We also assessed developmental trajectories of miR-218 expression in the PFC in females and find a rapid rise of expression in early adolescence, reaching adult-like levels by mid adolescence ($F(2, 23)=11.38$, $p=0.0004$, one-way ANOVA). This is in contrast to miR-218 expression in the male PFC, which increases gradually during early and mid adolescence until adulthood. These results suggest that miR-218 administered non-invasively in adolescence can have lasting impact on behavior. The timing and the direction of this effect, however may differ between males and females. Elucidating molecular mechanisms underlying adolescent PFC development and individual differences in susceptibility to stress could provide insight into the discovery of biomarkers of psychiatric risk.

Disclosures: A. Morgunova: None. **M. Giroux:** None. **G.A. Hernandez:** None. **C. Flores:** None.

Poster

P076. Mechanisms of Vulnerability

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P076.12

Topic: A.09. Adolescent Development

Support: NIH-NIGMS Grant-GM060507
NIH-NIDDK Grant-DK124727
NIH-NIDDK Grant-DK124727-01S1
NIH Grant-MD006988

Title: Neuropathological Signatures Connecting Early-Life Trauma to Compulsive Eating Behavior and Obesity

Authors: *P. ONTIVEROS-ANGEL[1], V. E. WILLIAMS[1], T. B. SIMON[1], F. SHARAFEDDIN[1], J. LOU[2], I. DELA PENA[3], C. VIET[4], M. POMPILUS[5], M. FEBO[5], J. D. FIGUEROA[1];
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Abstract: Background: Childhood trauma heightens the risk of severe weight gain and adult obesity. However, the neuropathological correlates of increased risk of obesity in individuals exposed to early-life trauma remain poorly understood. Preclinical findings from our laboratory support the notion that alterations to cortical structures that control top-down control of feeding behavior, may account for the added risk of adult obesity in individuals with early life trauma. While anxiety and stress-related disorders are at least twice as prevalent in women as men, the neurobiological basis of this discrepancy has not been well studied. The present study identifies sex-dependent neuroadaptations mediating the elevated risk of obesity, cognitive deficits, and aberrant feeding behaviors in adolescents exposed to an obesogenic diet and early life trauma. **Methods:** Male and female adolescent Lewis's rats (n=96, 48M, 48F) were exposed to a novel two-hit model of predator-based psychosocial stress (PSS) followed by intermittent access to a Western-like high-saturated fat diet (WD). Animals were fed for twelve weeks with Western Diet (WD, 41% kcal from fat) or a matched control diet (CD, 13% kcal from fat) and evaluated longitudinally for molecular and behavioral effects. Subsequently, animals were exposed to three cycles of an intermittent feeding paradigm to evaluate binge eating-like behaviors. In female rats, the estrus cycle was monitored to assess cyclicity. Brains were scanned using MRI techniques to identify microstructural changes due to consumption of WD and challenges to their allostatic load. **Results:** We found that female rats displayed binge eating-like feeding behavior when intermittently exposed to WD over three cycles of binge eating. This phenotype was associated with heightened anxiety-like behaviors in the elevated plus-maze and enhanced background anxiety. Interestingly, in female rats exposed to PSS, estrus cyclicity evaluation revealed dysregulated length and variability in stage frequency. In addition, anatomical changes were found in the brains of the rats that consumed WD and were exposed to PSS. **Conclusions:** Our findings demonstrate that early life stress and consumption of an obesogenic WD during adolescence heightens behavioral vulnerabilities associated with risk for anxiety and stress-

related eating disorders. Furthermore, our model recapitulates sex differences in the traumatic stress response and binge-eating behaviors, identifying sexual dysmorphisms and neuroadaptative links between early-life trauma and compulsive eating behaviors.

Disclosures: P. Ontiveros-Angel: None. **V.E. Williams:** None. **T.B. Simon:** None. **F. Sharafeddin:** None. **J. Lou:** None. **I. Dela Pena:** None. **C. Viet:** None. **M. Pompilus:** None. **M. Febo:** None. **J.D. Figueroa:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.01

Topic: B.08. Intrinsic Membrane Properties

Support: NIH RO1 MH115832

Title: Long-term inactivation of sodium channels as a mechanism of adaptation in CA1 pyramidal cells with implications for place cell firing

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Abstract: The hippocampus is involved in memory and spatial navigation. Many CA1 pyramidal cells function as place cells, increasing their firing rate when an animal traverses a specific place field in its environment. The dependence of CA1 place cell firing on position within the place field is asymmetric as shown by *in vivo* recordings. We investigated this asymmetry by injecting triangular depolarizing current ramps to approximate the spatially-tuned, temporally-diffuse depolarizing synaptic input received by these neurons while traversing a place field. Ramps were applied to rat CA1 pyramidal neurons *in vitro* (slice electrophysiology) and *in silico* (multi-compartmental NEURON model using several biophysically realistic morphologies). Under control conditions, CA1 neurons fired more action potentials at higher frequencies on the up-ramp versus the down-ramp, producing asymmetric, adapting voltage responses. This effect was more pronounced for dendritic compared to somatic ramps. Pharmacological blockers specific for K^+ currents that are known to underlie adaptation (SK, M-type or A-type) did not affect the asymmetry, measured as the ratio between the number of spikes on the up-ramp over those on the down-ramp. We incorporated into our model a five-state Markov scheme for $Na_V1.6$ channels with spatially-dependent parameters, in agreement with the literature showing that long-term inactivation increases along the dendrites with distance from

the soma. This spatial dependence was sufficient to explain the more pronounced adaptation in dendritic versus somatic ramps. Modeling two components of the delayed rectifier (K_v1 and K_v2) separately was essential because of the role of K_v2 channels in the control of the firing frequency. PKC activators like the phorbol ester Phorbol-12, 13 Dibutyrate (PDBu) are known to reduce Na_V channel long-term inactivation in CA1 neurons. *In vitro* application of PDBu removed attenuation in back-propagating spike amplitude during dendritic spike trains, consistent with decreased Na_V long-term inactivation. Moreover, PDBu greatly reduced adaptation, consistent with our hypothesized mechanism. Our synergistic experimental/computational approach shows that long-term inactivation of $Na_V1.6$ is the primary mechanism of adaptation in CA1 pyramidal cells, and it is strongly affected by phosphorylation, and therefore by neuromodulators. Since changes in adaptation control the position of the center of mass of the place field, modulation of $Na_V1.6$ long-term inactivation levels could affect the timing of place cell firing.

Disclosures: C. Upchurch: None. **C.L. Combe:** None. **C.J. Knowlton:** None. **C.C. Canavier:** None. **S. Gasparini:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.02

Topic: B.08. Intrinsic Membrane Properties

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement numbers 945539 (Human Brain Project SGA3)
Fenix computing and storage resources under the Specific Grant Agreement No. 800858 (Human Brain Project ICEI)
Swiss National Supercomputing Centre (CSCS) under project ID ich002

Title: On the dynamic modulation of spike threshold and depolarization baseline in mouse hippocampal CA1 pyramidal neurons

Authors: *R. MIGLIORE[1], D. BIANCHI[1], P. VITALE[1], M. GARAD[2], P. A. POUSINHA[3], H. MARIE[3], V. LESSMANN[2], M. MIGLIORE[1];
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Abstract: In this work we study an electrophysiological feature often observed in recordings from mouse CA1 pyramidal cells. It consists of a progressive increase in the spike threshold and in the depolarization baseline (i.e. the minimum value of the membrane potential between

successive action potentials, DBL) as a function of the somatic current injection. These effects are potentially important, because they can directly affect neurotransmitter release and, more generally, the efficacy of synaptic transmission. However, they have been ignored so far by experimentalists (probably because of the technical difficulty in measuring fast transient mechanisms during a stimulus) and modelers (because none of the current computational models is able to reproduce them). Here we present a model addressing this point. It suggests that these effects can be an indication of a significant dynamic change of the membrane properties occurring at the input location. The results suggest that the processes responsible for the spike threshold and DBL increase can be a local, stimulus-induced, dynamic alteration of local ion concentrations and permeabilities, and point to potentially important consequences for I/O and dendritic integration properties that should be considered by both modelers and experimentalists, if they occur under normal *in vivo* activity. Modelers implementing detailed computational models of neurons and networks subjected to strong inputs, without taking into consideration the effects, can miss low frequency components (in the θ range in our case) of membrane oscillations both at the soma and at dendritic synaptic locations. The former can alter the frequency content of the signal propagated to the rest of the network, the latter can unpredictably alter the induction of local synaptic plasticity. Experimentalists may want to reconsider the range of input currents used *in vitro* to assess the intrinsic neurons' excitability. Hippocampal CA1 pyramidal neurons of mice fire action potentials at an average frequency that is approximately 5-fold higher than in rats. If this activity can be considered physiologically plausible, it implies that the range of currents used to test neurons of other species needs to be extended to include strengths that allow to fire action potential at the same rate as for the mouse. In this case, and assuming that the mammalian's hippocampus has similar local ionic microenvironment at rest to the mouse, the model predicts that the same phenomena should also be observed for other species during inputs able to generate action potentials above approximately 40 Hz, with an extent of DBL that will depend on the specific neuron properties.

Disclosures: R. Migliore: None. **D. Bianchi:** None. **P. Vitale:** None. **M. Garad:** None. **P.A. Pousinha:** None. **H. Marie:** None. **V. Lessmann:** None. **M. Migliore:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.03

Topic: B.08. Intrinsic Membrane Properties

Support: CONACYT Grant CB-2016-281617
CONACYT Fellowship 727269

Title: Activation of dopamine D1-like receptors ameliorates decreased intrinsic excitability potentiation of CA1 pyramidal cells induced by neonatal blockade of NMDA receptors

Authors: *E. GRIEGO, E. J. GALVÁN;
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Abstract: Dysregulation of dopaminergic transmission combined with transient hypofunction of N-methyl-D-aspartate receptors (NMDARs) is a key mechanism that may underlie cognitive symptoms of schizophrenia. Therefore, we aimed to identify electrophysiologic alterations in animals neonatally treated with the NMDA receptor antagonist, MK-801 or with saline solution. Patch-clamp whole-cell recordings from MK-801-treated animals revealed altered passive and active electrophysiologic properties compared with CA1 pyramidal cells from saline-treated animals, including upregulation of the K^+ inward-rectifier conductance and fast-inactivating and slow/non-inactivating K^+ currents. Upregulation of these membrane ionic currents reduced the overall excitability and altered the firing properties of CA1 pyramidal cells. We also explored the capability of cells treated with MK-801 to express intrinsic excitability potentiation, a non-synaptic form of hippocampal plasticity associated with cognition and memory formation. CA1 pyramidal cells from animals treated with MK-801 expressed a diminished intrinsic excitability potentiation and had blunted synaptic potentiation. Interestingly, we found that activation of D1-like dopaminergic receptors with the agonist SKF-38, 393, partially restores the plastic capabilities of CA1 PCs. Our results offer a molecular and mechanistic explanation based on dysregulation of glutamatergic in addition to dopaminergic transmission that may contribute to the understanding of the cognitive deterioration associated with schizophrenia.

Disclosures: E. Griego: None. **E.J. Galván:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.04

Topic: B.08. Intrinsic Membrane Properties

Support: NIH grant R01-NS101356

Title: Plateau-generating and pacemaker neurons have similar ion channel subtypes with different voltage and kinetic properties

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Abstract: Some neurons express a plateau of activity greatly outlasting the stimulation, whereas others do not. The difference between these firing patterns is generally believed to be due to the

different expression of specific ion channels (e.g., persistent Na^+, and Ca^{2+}). However, an alternative possibility is that the same channels are expressed but they have different properties. To examine this issue, we compared two different plateau-generating neurons, B51 and B64, with a known pacemaking neuron, B8, in *Aplysia californica.* We found that all three neurons expressed a delayed K^+, A-type K^+, L-type and R-type calcium and preliminary data suggests all express the persistent Na^+ (Napp), and K_{Ca} was expressed in B8 and B51. Suprisingly, several channel properties were similar in neurons with vastly different firing properties. For example, the voltage dependency of activation of L-Type Ca^{2+} and R-Type Ca^{2+} was similar between B51, B64, and B8. The voltage dependency of activation and inactivation of the delayed K^+ was similar in B51, B64, and B8. Whereas other properties were dramatically different in neurons with similar firing properties. For example, a steeper voltage dependency and higher current density in the A-type K^+ in B51 compared to B64 and B8. The activation kinetics of the L-Type Ca^{2+} and R-Type Ca^{2+} was slower in B8 and B51 compared to B64. Conductance-based computational models will help explain how the different firing properties emerge from the unique ion channel properties and provide insights into how channel properties affect computations within a network.

Disclosures: C. Neveu: None. **J.H. Byrne:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.05

Topic: B.08. Intrinsic Membrane Properties

Support: NS R35111562
NS F32101832

Title: Mechanisms that regulate firing rate set points are cell type-specific

Authors: *N. TROJANOWSKI, G. TURRIGIANO;
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Abstract: To enable robust network stability in the face of dynamic sensory inputs and developmental changes, rodent neocortical pyramidal neurons actively maintain their mean firing rates within a target range. While this target range, or firing rate set point (FRSP), can span several orders of magnitude within a transcriptional cell type, individual pyramidal neurons reliably return to their own specific FRSP during prolonged activity perturbations. Thus, individual FRSPs are actively maintained. High and low FR neurons in rodent frontal cortex and hippocampus are differentially modulated by sleep states, suggesting that they are functionally distinct and may play unique roles in information storage and transmission. However, little is

known about the factors that generate this broad distribution of FRSPs observed across different cell types *in vivo*.
To gain selective access to high and low activity neurons, we used the permanent green-to red Ca^{2+}- and UV-dependent photoconversion of CaMPARI2 to label neurons in rodent visual cortex based on their mean activity levels. Red/green ratios following 30-minute *in vivo* photoconversion were lognormally distributed, mirroring *in vivo* firing rates, and neurons with greater *in vivo* photoconversion had higher firing rates *ex vivo*. During *ex vivo* photoconversion, neurons with higher firing rates underwent greater changes in CaMPARI2 red/green ratio. Thus, CaMPARI2 labeling is sensitive enough to detect differences in mean firing rates and differentiate between neurons with low and high FRSPs.
We next measured intrinsic and synaptic electrophysiological properties in high and low FR neurons in different cell types. Using *in vivo* and *ex vivo* photoconversion protocols, we found that high activity layer 4 (L4) pyramidal neurons had greater intrinsic excitability and received stronger inputs from other L4 pyramidal neurons, although there were no consistent differences in total E/I ratio between these neurons. In contrast, we then found that in L5 TLX-3-positive neurons (a subset of thin-tufted, regular spiking pyramidal neurons), high activity neurons received stronger excitatory input but had no increase in intrinsic excitability. Experiments using RNAseq to identify gene transcripts differentially expressed in high and low activity neurons are ongoing.
Together, our data demonstrate that intrinsic excitability and excitatory synaptic strength can be important contributors to the broad range of FRSPs observed within single cell types *in vivo*, though their relative importance in determining FRSPs of individual neurons differs across cell types.

Disclosures: N. Trojanowski: None. **G. Turrigiano:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.06

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant: R01AA028938
NIH Grant: R21NS108128
NIH Grant: R01AA025784

Title: Increased excitability of pyramidal neurons in the secondary motor cortex mediates cocaine relapse

Authors: *D. HUANG[1], S. IM[2], J. M. CIALDELLA[2], A. V. SALINAS[2], Y. Y. MA[1];
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Abstract: Drug-induced impairments to the medial prefrontal cortex (mPFC) contribute to persistence of drug craving and relapse. However, the cortical neuronal substrates underlying habitual drug-seeking behaviors remain unclear. Here, we explored the motor cortex, including the primary and secondary motor areas (M1 and M2), as well as the mPFC, including the infralimbic (IL), prelimbic (PrL), and anterior cingulate (AC) cortex. Sprague-Dawley (SD) rats, experienced with intravenous self-administration (IVSA) of saline or cocaine, 1 hour per day for 5 days, were tested *in vivo* or *in vitro* 1 or 45 days after withdrawal (WD1 or WD45). Cocaine-experienced rats showed significant increases of drug seeking behaviors on WD 45 vs. WD1. Whole-cell patch clamp recordings of pyramidal neurons in layer 2/3 (denoted L2) from cocaine-experienced rats demonstrated a transiently decreased excitability in the PrL and M2 on WD1, compared to that in saline rats. Interestingly, only the M2 from cocaine-experienced rats showed an increased excitability on WD45. No differences of excitability between saline vs. cocaine rats were detected in layer 5 (denoted L5) in any of the 5 cortical areas tested. $GABA_A$ receptor agonist, muscimol, dose-dependently (0.1 mM, 0.3 mM and 10.mM) decreased the excitability of L2 pyramidal neurons in the M2 on WD 45. *In vivo* bilateral microinjection of muscimol primarily targeting L2 in the M2 attenuated cocaine-seeking behaviors on WD45. In addition, chemogenetic inhibition by activating Gi-coupled DREADD receptor primarily expressed in L2, but not L5, in the M2 attenuated cocaine-seeking behaviors on WD45. Neither pharmacological nor chemogenetic inhibition approaches altered general locomotor activity. Our results demonstrate that cocaine IVSA induces differential short-term and prolonged excitability changes in the motor cortex and mPFC. Importantly, the increased excitability in the M2, particularly in L2, as a prolonged effect after cocaine IVSA, could be a novel target in preventing drug-relapse during withdrawal.

Disclosures: D. Huang: None. **S. Im:** None. **J.M. Cialdella:** None. **A.V. Salinas:** None. **Y.Y. Ma:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.07

Topic: B.08. Intrinsic Membrane Properties

Support: NIH grant 5R01NS102871 (MPI: Hochman, Prinz)

Title: Sympathetic postganglionic neurons as a potential locus of modulation and plasticity in the sympathetic pathway

Authors: *N. H. CHANG[1], M. L. MCKINNON[1], S. HOCHMAN[2], A. A. PRINZ[1];
[1]Dept. of Biol., [2]Dept. of Physiol., Emory Univ., Atlanta, GA

Abstract: Thoracic sympathetic postganglionic neurons (tSPNs) in paravertebral chain ganglia are the final autonomic output neurons regulating vascular and thermoregulatory systems. tSPNs were long thought to act as relays of the spinal signals, however, we previously used computational modeling to show that tSPNs integrate inputs more effectively than presumed. Important questions are whether tSPN membrane properties: (i) are tuned to optimally process synaptic inputs, (ii) represent a locus for behavioral state-dependent modulation, and (iii) undergo compensatory homeostatic changes in excitability due to long-term alterations in central preganglionic synaptic drive. We address these questions in a tSPN mouse model. Its membrane currents include: a fast sodium current I_{Na}; a low-threshold calcium current I_{CaL}; a delayed-rectifier potassium current I_{Kd}; a fast transient potassium current I_A; a slow and non-inactivating potassium current I_M; and a calcium-dependent potassium current I_{KCa}; a hyperpolarization-activated inward current I_H; and a leak current I_{leak}. To simulate synaptic input from the spinal cord, we provide the tSPN model with excitatory synaptic conductance waveforms that match measurements from mouse tSPNs in number, synapse strength, and presynaptic firing pattern. We find that this synaptic input results in an increase of synaptic gain, confirming that tSPNs may be able to integrate and boost spinal inputs. We next individually vary the maximal conductance for each membrane current from its canonical value and observe that: 1) Varying the conductances for I_{Kd}, I_A, and I_H has little effect on synaptic gain. These currents therefore may not be effective targets for modulation or plasticity. 2) Increasing the conductance for I_{Na} increases synaptic gain, as expected for an inward current. 3) Increasing the conductance for I_M, I_{KCa}, and I_{CaL} reduces synaptic gain. For the I_M and I_{KCa} this occurs because they are outward currents, thus increasing their conductance decreases tSPN excitability. I_{CaL} is an inward current but has a negative effect on synaptic gain because it indirectly reduces excitability via increasing the outward current I_{KCa}. Our simulations implicate the tSPN membrane currents I_{Na}, I_M, I_{KCa}, and I_{CaL} as factors that may determine tSPN excitability and synaptic gain, with I_{Na} as a positive regulator and I_M, I_{KCa}, and I_{CaL} as negative regulators. These currents may be suitable targets for plasticity and modulation of signal integration in the thoracic sympathetic pathway, including in therapeutic approaches that aim to control dysfunction in autonomic drive (e.g. after spinal cord injury).

Disclosures: N.H. Chang: None. **M.L. McKinnon:** None. **S. Hochman:** None. **A.A. Prinz:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.08

Topic: B.08. Intrinsic Membrane Properties

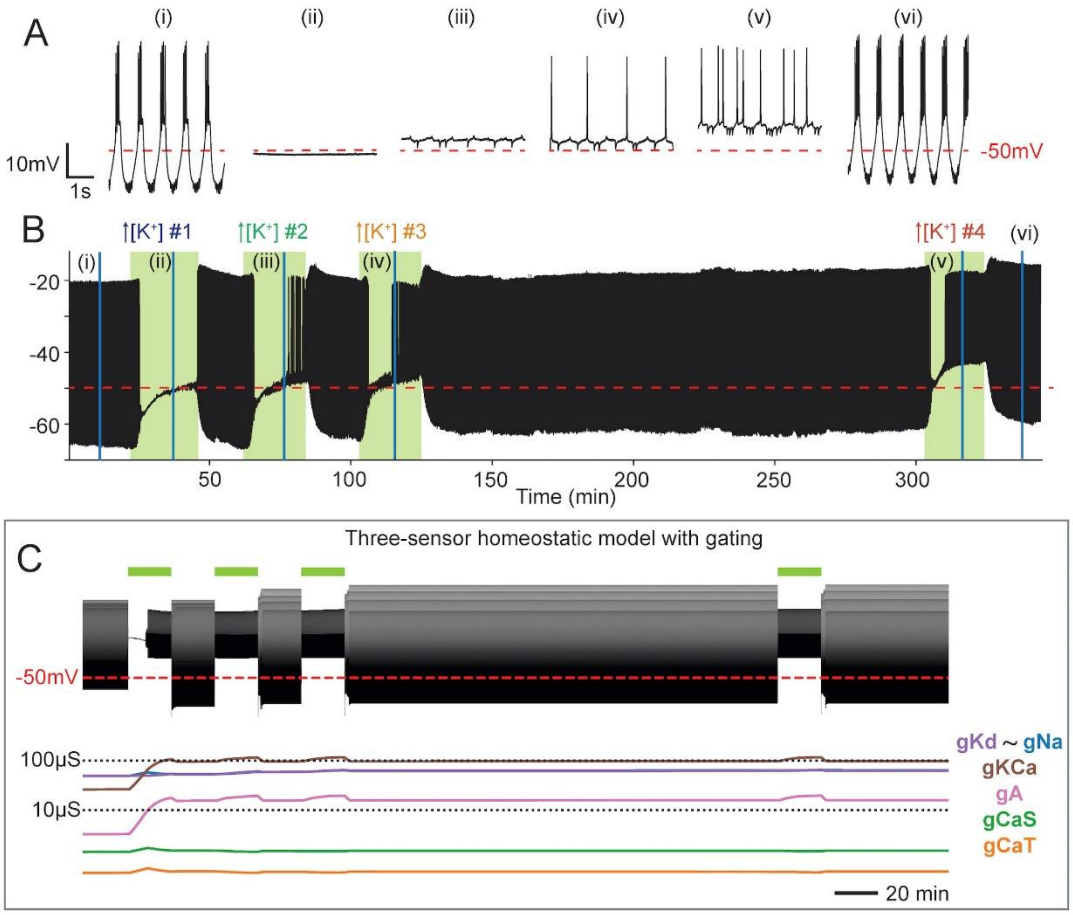
Support: MH 46742

NIH Grant R35 NS097343
NIH Grant F31-NS113383

Title: A model of rapid homeostatic plasticity accounts for hidden, long-lasting changes in a neuronal circuit after exposure to high potassium

Authors: *M. C. P. RUE, L. M. ALONSO, E. E. MARDER;
Brandeis Univ., Waltham, MA

Abstract: Neural circuits are remarkable in their ability to simultaneously function reliably and flexibly adapt to changes in their environment. In addition, circuits that perform the same physiological function can be built with different underlying parameters. It remains unclear how neural circuits can balance adaptation to perturbation with physiological stability. To study this question, we used the pyloric circuit within the stomatogastric ganglion of the crab *Cancer borealis*, a neural circuit with a long history of both experimental and computational studies. Here we present the effects of one type of perturbation, high extracellular potassium concentration, on both biological neurons and computational models. Pyloric neurons of the crab stomatogastric ganglion (STG) initially become quiescent, then recover spiking activity in high potassium saline (2.5x[K^+], He et al. 2020). The neurons retain this adaptation and recover more rapidly in subsequent high potassium applications, even after hours in control saline (Figure A,B). We constructed a novel activity-dependent computational model that qualitatively captures the main observations in our experiments. In this model, regulation of conductances is gated on and off depending on how far the neuron is from its target activity. This allows the model neuron to retain a memory of past perturbations even after it returns to its target activity in control conditions (Figure C). When these rules are implemented, models of bursting neurons adapt to the high potassium perturbation and maintain this robustness upon subsequent applications. Finally, we show that in biological neurons adaptation to high potassium can be erased by presenting a new challenge such as low potassium saline (0.4x[K^+]). Thus, perturbation, followed by recovery of normal activity, can hide cryptic changes in neuronal properties that are only revealed by subsequent perturbations.



Disclosures: M.C.P. Rue: None. **L.M. Alonso:** None. **E.E. Marder:** None.

Poster

P126. Dendritic Integration, Excitability, and Plasticity

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P126.09

Topic: B.08. Intrinsic Membrane Properties

Support: Israel Science Foundation, 1496_2016
Human Brain Project, 604102
Edmund and Lily Safra Center for Brain Sciences

Title: Fast depolarizing events triggered by synaptic inputs suggest activation of intrinsic regenerative currents in axonal spines

Authors: *N. VRIELER[1], Y. LEFLER[2], O. AMSALEM[3], N. MEDVEDEV[4], K. VASO[1], A. GURKAN[4], I. SEGEV[1], M. Y. UUSISAARI[4], Y. YAROM[1];
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Abstract: Dendritic spines have received a lot of attention in the context of understanding the structure and plasticity of neuronal processing, while spines on axons are relatively rarely studied even though they are not uncommon (see for example Amaral 1978; Williams, Goldman-Rakic and Leranth 1992; Miller, Chiaia and Rhoades 1990). Spine heads on axons innervated by excitatory inputs - as have been described in the Inferior Olive (IO) of cat (de Zeeuw et al. 1990) - would appear to be in a prime position for activating the neuron's output with high priority. Nevertheless, the possible functional roles of this input-output configuration have not yet been investigated. In this work, we present evidence that excitatory inputs innervating IO neuron axonal spine heads can trigger particularly large and fast sub-threshold depolarizations (with amplitudes up to 30mV and rise-time 0.3 - 1.5ms, comparable to action potentials). Specifically, we hypothesize that these events result from 'amplification' of the incoming inputs by intrinsic regenerative currents located in the axonal spine heads. Large, fast depolarizations occur spontaneously in mouse IO neurons, and are especially intriguing because the amplitude distribution of these events usually shows a distinct grouping while the waveforms of the amplitude-normalized events within a single neuron are identical. Using *in vitro* patch-clamp experiments we show that these events are eliminated by pharmacological blockade of glutamatergic transmission, and that they can be activated by optogenetic stimulation of specific groups of axons that innervate the IO. By simulating various possible sources of these events on a reconstructed IO neuron morphology in NEURON, we show that these properties are best explained by assuming that the events originate as intrinsic regenerative currents in axonal spine heads. This is further supported by electron microscopy data demonstrating the existence of spines on the axons of mouse IO neurons, and by additional anatomical experiments combining immunofluorescence and super-resolution confocal microscopy methods. Taken together, our results indicate that the intrinsic physiological properties of axonal spines may make them especially significant in the study of how spines contribute to neural computation.

Disclosures: N. Vrieler: None. **Y. Lefler:** None. **O. Amsalem:** None. **N. Medvedev:** None. **K. Vaso:** None. **A. Gurkan:** None. **I. Segev:** None. **M.Y. Uusisaari:** None. **Y. Yarom:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.01

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

Support: NIH Grant U54 AG054349

Title: TREM2*R47H^{em1Aduci}; 5xFAD mice display sex-specific differences in plaques and plaque-associated microglia

Authors: *K. M. TRAN[1,2], S. KAWAUCHI[3], S. FORNER[1], D. JAVONILLO[1], C. DA CUNDA[1], J. PHAN[1], K. TRAN[1], N. REZAIE[4], H. LIANG[1], A. MORTAZAVI[5], A. TENNER[1,2,6], F. LAFERLA[1,2], G. MACGREGOR[3,5], K. GREEN[1,2];
[1]Univ. of California, Irvine, Inst. for Memory Impairments and Neurolog. Disorders, Irvine, CA; [2]Univ. of California Irvine, Neurobio. and Behavior, Irvine, CA; [3]Univ. of California, Irvine, Transgenic Mouse Facility, Irvine, CA; [4]Univ. of California, Irvine, Mathematical, Computat. and Systems Biol., Irvine, CA; [5]Univ. of California, Irvine, Dept. of Developmental and Cell Biol., Irvine, CA; [6]Univ. of California Irvine, Mol. biology and Biochem., Irvine, CA

Abstract: Genome-Wide Association Studies revealed *Triggering receptor expressed on myeloid cells 2* (*TREM2)* R47H mutation as one of the strongest genetic risk factors for late-onset Alzheimer's Disease (AD). In the brain, TREM2 is a transmembrane receptor expressed exclusively by myeloid cells. Many current TREM2 R47H mouse models have observed evidence of cryptic splicing products of the mutant allele, resulting in artefactually reduced protein product. Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD) consortium at University of California - Irvine has developed TREM2*R47H^{em1Aduci} mouse model (TREM2^{R47H}) where *Trem2* ^{em1Aduci} allele is expressed at a level similar to the wild-type *Trem2* allele, with no evidence of cryptic splicing products from the mutant allele. In this experiment, we set out to investigate this mouse model on C57BL/6J background and the effect the variant has on AD pathology when crossed with 5xFAD mice, generating four groups – WT, TREM2^{R47H} homozygous, 5xFAD, and 5xFAD x TREM2^{R47H} homozygous. Coronal brain slices of 4-month-old mice (n=5/sex/genotype) were immunolabeled with Thioflavin-S for dense-core plaques and ionized calcium binding adaptor molecule 1 (IBA1) for microglia. Analyzing subiculum and cortical confocal images revealed sex-related differences in dense-core Aß plaque burden and size in 5xFAD x TREM2^{R47H}, where female 5xFAD x TREM2^{R47H} mice exhibited increased plaque load compared to 5xFAD mice. In males, stark reductions in plaque load were observed. Examination of IBA1^{+} microglia revealed significant reductions in microglia volume in both TREM2^{R47H} and 5xFAD x TREM2^{R47H} mice compared to their controls. Notably, there was a significant impairment of plaque-microglia interaction in 5xFAD x TREM2^{R47H}, where a decrease in microglia and plaque colocalization was observed. Moreover, we found a significant increase in plaque-associated neuritic damage in 5xFAD x TREM2^{R47H} when immunolabeled with Lysosome-associated membrane glycoprotein 1 (LAMP1) for neuritic dystrophy. Gene expression analyses again found sex-specific effects, but collectively microglial gene expression responses to pathology were unaltered except for *Itgax (*or *Cd11c)* and *Cd74*, which failed to induce in 5xFAD x TREM2^{R47H} mice. Immunohistochemistry confirmed a reduction in both CD11C^{+} and CD74^{+} plaque associated microglia in 5xFAD x TREM2^{R47H} mice compared to 5xFAD mice. Collectively, these results show the effects of the *Trem2^{R47H}* missense mutation on plaque development and downstream pathology and highlight sex differences.

Disclosures: K.M. Tran: None. **S. Kawauchi:** None. **S. Forner:** None. **D. Javonillo:** None. **C. da Cunda:** None. **J. Phan:** None. **K. Tran:** None. **N. Rezaie:** None. **H. Liang:** None. **A. Mortazavi:** None. **A. Tenner:** None. **F. LaFerla:** None. **G. MacGregor:** None. **K. Green:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.02

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

Support: USAMRAA award # W81XWH2010665
Purdue Research Foundation Award, 60000019
Purdue Integrative Data Science Institute award
Indiana Clinical and Translational Sciences Institute grant # UL1TR002529
Ralph W. and Grace M. Showalter Research Trust award
Jim and Diann Robbers Grant for New Investigators award
Purdue University Center for Cancer Research funded by National Institutes ofHealth grant # P30 CA023168

Title: Microglial lipid droplet accumulation and dysfunctional state is induced by amyloid-β exposure in neurodegeneration

Authors: P. PRAKASH[1], P. MANCHANDA[1], P. R. WIJEWARDHANE[1], C. E. RANDOLPH[1], A. D. DUNHAM[1], J. FINE[1], E. THAYER[1], C. R. FERREIRA[2], ***G. CHOPRA**[1];
[1]Chem., [2]Bindley Biosci. Ctr., Purdue Univ., West Lafayette, IN

Abstract: Glial cell phagocytosis is essential for the regulation of brain function during health and disease. The onset of Alzheimer’s disease (AD) has been linked to the accumulation of amyloid β (Aβ); however, the changes to glial phagocytic function affected by Aβ remain poorly understood. Previous studies have indicated that microglia in aged brains accumulate lipid droplets (LDs) and have a proinflammatory and defective phagocytic phenotype. However, the effect of Aβ on microglial lipidome and related function in neurodegeneration remains uncharacterized to date. We show that microglia from wild-type (WT) mice exposed to Aβ in culture induces upregulation of triacylglycerols (TAGs), a major component of LD, suggesting a possible role of Aβ exposure in LD formation. We used unbiased lipidomics and metabolomics to characterize 1500 lipid species categorized into 10 main classes and around 700 metabolites to identify the Aβ-associated molecular changes in microglia. Further, acutely isolated microglial cells from AD mouse models (5xFAD) show more LD accumulation compared to age-matched WT controls. These LDs comparisons of 5xFAD vs. WT were significantly higher in female mice compared to males and showed an age-dependent effect for LD accumulation. Additionally,

the 5xFAD microglia showed lower phagocytosis of Aβ, with most of the "phagocytic defect" exhibited by LD+ cells. To characterize phagocytosis, we have previously developed a human Aβ1-42 analog ($A\beta^{pH}$) that exhibits green fluorescence upon internalization into the cellular acidic phagosomes. This allowed us to image, for the first time, glial uptake of $A\beta^{pH}$in real-time in live animals. We show that the dysfunction in $A\beta^{pH}$ phagocytosis by 5xFAD microglia is a result of LD accumulation, perhaps due to chronic exposure of Aβ *in vivo*. The phagocytic dysfunction is rescued in the presence of a LD-reducing compound. We have characterized the pathway of TAG synthesis leading to LD formation and propose an Aβ-dependent LD accumulation and phagocytic dysfunction model in microglial cells. We hypothesize that increased LDs in microglia is a cellular protective mechanism to circumvent toxicity during chronic inflammation that leads to its dysfunctional state and disease progression. Characterizing the lipidome and metabolome of different glial cell subpopulations that "eat" (or do not "eat") Aβ *in vivo* will elucidate molecular mechanisms of phagocytic function and dysfunction during neurodegenerative disease and chronic inflammation.

Disclosures: P. Prakash: None. **P. Manchanda:** None. **P.R. Wijewardhane:** None. **C.E. Randolph:** None. **A.D. Dunham:** None. **J. Fine:** None. **E. Thayer:** None. **C.R. Ferreira:** None. **G. Chopra:** Other; Director of Merck-Purdue Center for Measurement Science.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.03

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

Support: Research and Education Program, a component of the Advancing a Healthier Wisconsin endowment at the Medical College of Wisconsin
NIH Grant MH119105

Title: Effect of WNT pathway inhibitors on synapse loss induced by soluble Aβ oligomers

Authors: *D. NIEVES TORRES, S. SKAAR, S. H. LEE;
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Abstract: The loss of excitatory synapses is a hallmark of Alzheimer's Disease (AD) resulting from the formation of amyloid beta (Aβ) plaques. Several neurodegenerative diseases have been heavily associated with disruption of WNT signaling. A WNT inhibitor Dickkopf-related protein-1 (Dkk-1) is thought to be the downstream mediator of Aβ but the detailed molecular mechanisms that trigger the Aβ-induced synapse degeneration have yet to be elucidated. Proline-rich 7 (PRR7) is a novel WNT inhibitor that upon secretion via exosomes, has been found to trigger synapse loss. To investigate the role of PRR7 in Aβ-induced synapse loss, exosomal

secretion of PRR7 upon application of soluble Aβ oligomers (ADDLs) in cultured hippocampal neurons was investigated. Interestingly, protein levels of PRR7 in exosomes were drastically increased in neurons exposed to ADDLs, suggesting that increased exosomal secretion of PRR7 is involved in Aβ-induced synapse loss. Notably, we found that Dkk-1 also increased the exosomal secretion of PRR7. Administration of a Dkk-1 inhibitor, WAY-262611 (WAY) to hippocampal neurons effectively blocked ADDLs-induced loss of excitatory synapses, quantified by immunocytochemistry analysis. These results suggest that PRR7 might be the downstream mediator of Aβ and Dkk-1 in synapse degeneration found in AD. Overall, these findings suggest that impeding these WNT pathway inhibitors can promote synapse maintenance in an AD model.

Disclosures: D. Nieves Torres: None. **S. Skaar:** None. **S.H. Lee:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.04

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

Support: Cure Alzheimer's
Swedish Alzheimerfonden
Alzheimer's Research UK TVPG 72499

Title: Examining amyloid-beta aggregation dynamics in amyloid precursor protein knock-in mice using stable isotope labelling kinetics and imaging mass spectrometry, and the role of microglia

Authors: *K. M. STRINGER[1,3], W. MICHNO[4], J. WOOD[1,3], J. GE[3], S. KOUTARAPU[3], K. VITANOVA[1], T. TRIPATHI[1], D. CUMMINGS[1], F. EDWARDS[1], J. HANRIEDER[3,2];
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Abstract: Aims: Amyloid beta (Aβ) plaque deposition is one of the major pathological features of Alzheimer's disease (AD); however, the mechanism by which this occurs remains unclear. Microglia, the immune cells of the brain, have been shown to interact with plaques but their significance to Aβ deposition is unknown. This project aims to assess Aβ deposition in detail both in amyloid precursor protein (APP) knock-in mice, and also assess the interaction with microglia. **Methods:** Specific aggregation dynamics of individual Aβ species and plaque-associated lipids were analysed in knock-in mouse models of Alzheimer's disease (APP^{NL-G-F} and APP^{NL-F}) at a nanometer level using a novel chemical imaging paradigm termed 'iSILK', involving stable isotope labelling *in vivo* followed by MALDI imaging mass spectrometry.

Microglia morphology and localisation in relation to plaque type are additionally characterised by hyperspectral imaging. **Results:** In APP^{NL-G-F} mice, Aβ1-42 is deposited first and localises to the core of plaques, followed by further recruitment of Aβ1-42 and secondary production of Aβ1-38. In APP^{NL-F} mice, heterogeneity in the morphology and peptide composition of plaques may change microglia behaviour and density compared with in APP^{NL-G-F} mice. The role of Trem2 in the interaction of microglial with plaques will be further analysed by introduction of the Trem2R47H GWAS risk factor mutation to the APP^{NL-F} mice.**Conclusions:** iSILK can be used to distinguish between initial Aβ seeds and later deposition depending on the deposition of labelled isotopes. This furthers our mechanistic understanding of the early production and propagation dynamics of Aβ pathology.

Disclosures: K.M. Stringer: None. **W. Michno:** None. **J. Wood:** None. **J. Ge:** None. **S. Koutarapu:** None. **K. Vitanova:** None. **T. Tripathi:** None. **D. Cummings:** None. **F. Edwards:** None. **J. Hanrieder:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.05

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

Support: U54 AG054349
U54 AG054345

Title: Abca7*v1599m^{Em1aduci} variant in 5xfad mice mediates differences in amyloid-beta pathology and reactive gliosis

Authors: *D. JAVONILLO[1], L. A. HOUCHIN[1], S. KAWAUCHI[2], *J. PHAN[3], C. DA CUNHA[3], K. TRAN[3], G. MILINKEVICIUTE[3], A. J. TENNER[4], F. M. LAFERLA[3], G. R. MACGREGOR[4], K. N. GREEN[3];
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Abstract: In several large genome-wide association studies (GWAS), genetic polymorphisms of ATP-binding cassette transporter subfamily A member 7 (Abca7) have been identified as a risk factor for late-onset Alzheimer’s Disease (LOAD). ABCA7 is a member of a family of ATP-binding cassette (ABC) transporters and functions as a transmembrane protein mediating the release of cholesterols and phospholipids from cellular membranes. Previous studies have also proposed its role in mediating phagocytic clearance of amyloid beta (Aβ) oligomers within macrophages in the brain. To study the effects of ABCA7 on the development of AD relevant

pathologies we introduced an equivalent coding sequence change that has been identified as a risk variant for LOAD (V1613M) into the C57BL/6 mouse genome using CRISPR/Cas9 (V1599M). We set out to characterize the ABCA7^{V1599M} variant and investigate its impact on AD pathology when crossed with 5xFAD transgenic mice, generating four distinct groups: WT, ABCA7^{V1599M} homozygous, 5xFAD, and 5xFAD x ABCA7^{V1599M} homozygous. Histological staining using Thioflavin-S on coronal brain sections from 4-month-old mice (n=5-6 mice/sex/genotype) revealed significantly reduced burden of dense-core Aβ plaques in confocal images of both subiculum and visual cortical regions of 5xFAD x ABCA7^{V1599M} mice compared to 5xFAD mice. Biochemical analysis mirrored these findings and demonstrated stark reductions of both soluble and insoluble Aβ40 and Aβ42 levels within these same brain regions. To examine reactive gliosis to plaque burden, we immuno-stained for ionized calcium binding adaptor molecule 1 (IBA1) and glial fibrillary protein (GFAP) to visualize microglia and reactive astrocytes, respectively. Confocal analysis revealed reduced densities of both IBA1+ microglia and GFAP+ reactive astrocytes within subiculum and cortical regions of 5xFAD x ABCA7^{V1599M} mice compared to 5xFAD mice. We also found reduced plaque-associated neuritic damage in 5xFAD x ABCA7^{V1599M} mice upon immuno-staining for lysosome-associated membrane glycoprotein 1 (LAMP1) to examine neuritic dystrophy. Together, these results characterize the effects of the *Abca7^{em1Aduci}* allele and its ABCA7^{V1599M} missense mutation on 5xFAD-mediated pathology, specifically in Aβ plaque development, glial morphology, and neuritic damage. Future studies would investigate whether Aβ pathologies are delayed or exacerbated in older mice expressing the *Abca7^{em1Aduci}* mutant allele.

Disclosures: D. Javonillo: None. **L.A. Houchin:** None. **S. Kawauchi:** None. **J. Phan:** None. **C. da Cunha:** None. **K. Tran:** None. **G. Milinkeviciute:** None. **A.J. Tenner:** None. **F.M. LaFerla:** None. **G.R. MacGregor:** None. **K.N. Green:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.06

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

Support: NIH R01 AG061151
NIH R01 AG055523
Cure Alzheimer's Fund

Title: Increased APP Gene Dose Induces Astrocyte Activation and Early Endosome Enlargement in a Down Syndrome Model

Authors: *R. ALBAY, A. H. BECKER, M. SAWA, C. OVERK, W. C. MOBLEY; Neurosciences, UCSD, La Jolla, CA

Abstract: Alzheimer's disease (AD) is the most common irreversible, progressive cause of dementia, which has become a social and economic issue. Down syndrome (DS) is caused by an extra copy of chromosome 21 (HSA21) and is believed to be linked to AD by the triplication of the amyloid precursor gene (*APP),* which is encoded on chromosome 21. Furthermore, an extra copy of *APP* is essential for the clinical presentation of AD in DS (AD-DS) and provides the basis for the *APP* gene does hypothesis. Here, we investigate the *APP* gene dose hypothesis by crossing a mouse model of HSA21 trisomy (Dp16) with an *APP* knockout model (m*APP-/-*) to generate a Dp16 mouse with one copy of the *APP* gene removed: Dp16(m*APP*++-). Glial cells and neuroinflammation are receiving increasing levels of recognition with respect to the biology of AD. Astroglia are involved in several processes both in the developing and mature brain; we asked whether *APP* gene does influences astroglia activation. To assess the possibility that astrocyte activation would characterize the young adult Dp16 brain, we examined the entorhinal cortex (EC) and medial septum (MS) of mice aged 4- and 9-months. Immunohistochemistry probing the EC show an elevated level of glial fibrillary acidic protein (GFAP) immunoreactivity (IR) in both parenchyma tissue and near the vasculature of the Dp16 mouse brain. Stereological analysis revealed a significant increase in the number of $GFAP^+$ astrocytes in Dp16 vs Dp16 (m*APP*++-) mice at both 4 and 9 months. In the MS, we observed no differences in $GFAP^+$ IR and astrocyte number between Dp16 and Dp16 (m*APP*++-) at 4 months, but a significant increase in $GFAP^+$ astrocytes at 9 months, suggesting age- and region-specific differences in *APP* pathology seen in DS. Dysregulation of early endosomes, with increased recruitment of Rab5 and enlargement, is an early feature of DS and is consistently present DS-AD and AD. To explore the role of *APP* gene does in this phenotype we asked if changes could be detected in 4- and 9-month-old mice. We again probed the EC and MS and compared 2N mice to Dp16 and Dp16 (m*APP*++-) mice. Tissue sections were stained with an antibody to Rab5 and random fields of the EC or MS were examined by fluorescent confocal microscopy and computer-aided morphometric analysis. Early endosomes with Rab5 IR were enlarged in the Dp16 with respect to 2N and Dp16 (m*APP*++-) as early as 4-months of age. In summary, studies in the Dp16 mouse demonstrated the presence of degenerative hallmarks associated with AD and AD-DS. In the case of GFAP and Rab5, the phenotype was normalized by reducing the m*APP* gene dose; providing evidence that increased m*APP* gene dose is necessary for several neurogenerative phenotypes in this model of AD-DS.

Disclosures: R. Albay: None. **A.H. Becker:** None. **M. Sawa:** None. **C. Overk:** None. **W.C. Mobley:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.07

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

Support: RFAG042189
1F31NS118970-01A

Title: Plasma from Alzheimer's Disease Patients Disrupts Astrocyte Mitochondrial Function

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Abstract: Alzheimer's disease (AD) is the most frequent cause of dementia characterized by a progressive decline in cognitive functions. Sex differences have been well documented in AD with a higher prevalence and higher rates of disease progression in women as compared to men. Cellular senescence of astrocytes and disturbances in the brain-gut axis may significantly contribute to the pathogenesis of neurodegenerative disorders. Recent evidence indicates that the gut is altered under AD disease conditions. Increased gut permeability dysregulates blood brain barrier (BBB) permeability, exposing astrocytes to circulating elements that could elicit a senescent phenotype. We obtained human plasma samples from the TARCC cohort and organized them into 24 groups based on their sex (male and female), race (Non-Hispanic white or Mexican American), diagnosis (probable AD, mild cognitive impairment or control) and Type 2 diabetes diagnosis. *In vitro* male and female human astrocyte cultures were treated with plasma from sex-matched AD and control groups. Treatment with the plasma significantly altered mitochondrial respiration in both male and female astrocytes, with AD plasma-treated cells experiencing significantly greater levels of oxygen consumption relative to control and vehicle groups. Furthermore, treatment with sodium butyrate and indole-3-carbinol, two gut metabolites commonly thought to be neuroprotective, evoked sex-specific responses in oxygen consumption rate after oxidative stress. Male astrocytes demonstrated elevated oxygen consumption in response to sodium butyrate treatment, while female astrocytes increased oxygen consumption in response to indole-3-carbinol treatment. Moreover, female astrocytes showed reduced production of ATP via glycolysis when treated with AD plasma as compared to control and vehicle treatment, resulting in a total decrease of ATP produced. Together these data indicate that exposure to the AD plasma triggers a sex-specific senescent phenotype in astrocytes that alter mitochondrial respiration, perhaps due to exposure to increased level of toxic metabolites circulating in AD plasma. In the future, studies that examine the development of astrocyte senescence will be an attractive target for the development of novel therapeutic agents to AD.

Disclosures: T. Branyan: None. **Y. El-Hakim:** None. **R.C. Barber:** None. **F. Sohrabji:** None.

Poster

P201. Effects of Amyloid Beta on Glial Function

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P201.08

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

Support: UCR start-up funds
NIH Grant MH087332
NIH Grant MH104131
NIH Grant MH105330
NIH Grant DA052209

Title: Neuroinflammatory pathways shared between Alzheimer's disease and neuroHIV

Authors: *I. S. HARAHAP-CARRILLO[1,2], D. OJEDA JUÁREZ[1,3], M. KAUL[1];
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Abstract: People living with HIV can develop HIV-associated neurocognitive disorders (HAND), which range from asymptomatic neurocognitive impairment to full-blown dementia. The neuropathological features of HIV infection of the brain (neuroHIV) are referred to as encephalitis (HIVE), including astrogliosis, microgliosis, and synaptic and dendritic loss. Interestingly, increased amyloid-beta (Aβ)1-42 and phosphorylated Tau has been observed in the cerebrospinal fluid of HAND patients but are considered hallmarks of Alzheimer's disease (AD), while AD brains also show an increase in microglial and astrocytic activation. Due to these similarities, we aim to identify potential mechanistic crossover between these neurodegenerative disorders. We analyzed the AD mouse model, J20, which overexpresses the mutated human amyloid precursor protein (APP) and compared it to a model of neuroHIV/HAND, HIVgp120tg mice. In the cortex (layer III) and hippocampus (CA1) of the J20 mice (12-month-old), we observed a loss in the presynaptic terminal marker synaptophysin, and an increased number of Iba1+ microglia. Sexual dimorphism was observed for the loss of synaptophysin in the hippocampus and the increased RNA expression of LCN2 in the cortex (higher in the female J20 mice). Moreover, protein expression of GFAP was increased in the cortex and hippocampus of J20 mice. Signaling pathways playing an integral role in HAND/HIVE seem to also be affected in the J20 mice, with increased activation of p38 MAPK and a trend of increased activation of ERK1/2 in the cortex. Overall, our findings in the AD and neuroHIV mouse model suggest at least a partial overlap in the activation of neuroinflammatory pathways in both disorders, and could reveal new potential targets for the treatment of neuroHIV/HAND and/or AD.

Disclosures: I.S. Harahap-Carrillo: None. **D. Ojeda Juárez:** None. **M. Kaul:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01EY029913

Title: Soluble and membrane-bound isoforms of fractalkine differentially regulate microglia activation and vascular damage in the diabetic retina

Authors: *D. RODRIGUEZ[1], K. A. CHURCH[1], A. N. PIETRAMALE[1], S. M. CARDONA[1], D. VANEGAS[1], K. R. NASH[2], A. E. CARDONA[1];
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Abstract: Diabetic retinopathy (DR) is a microvascular complication of *diabetes mellitus* and a leading cause of blindness worldwide. Hallmarks of DR include loss of neurons, activation of resident phagocytes, compromised vasculature, and angiogenesis. Inflammation caused by microglia exacerbates the retinal integrity, resulting in glial and neuronal cell dysfunction. The microglia-neuronal crosstalk mediated by CX3CR1/FKN signaling provides a neuroprotective environment in several diseases including DR. However, CX3CR1 polymorphic variants (V249I and T280M) present in about 20-30% of the population, decrease FKN binding affinity to CX3CR1, and their role in microglia function in the retina during diabetes is unknown. Moreover, FKN exists as a soluble chemokine and as a membrane-bound (mFKN) adhesion molecule. When cleaved by a disintegrin metalloproteinase (ADAM) protein, the soluble form (sFKN) is released to exert chemoattractant properties. Previous studies show that 1) diabetic CX3CR1-KO and FKN-KO mice display worse retinal pathology, and 2) intra-vitreal administration of recombinant FKN to diabetic FKN-KO mice rescued vascular pathology. However, the mechanisms by which mFKN and sFKN regulate retinal function are still unknown. We hypothesize that during diabetes, the over-expression of sFKN using recombinant adeno-associated viruses (rAAVs) will prevent vascular and neuronal damage, compared to mFKN. To test this hypothesis, rAAVs expressing either mFKN or sFKN were delivered to FKN-KO retinas. FKN protein levels, microglial activation, fibrinogen leakage, neuronal densities, and upregulation of pro-inflammatory mediators were compared in diabetic and non-diabetic controls. We validated the expression of FKN levels in the brain and retinal protein extracts in mice that received rAAV vectors expressing mFKN or sFKN. Immunofluorescence and high-resolution confocal imaging shows that sFKN can minimize microglial activation, reduce fibrinogen deposition, and rescue neuronal loss, compared to mice administered rAAV-mFKN. In conclusion, our data suggest that rAAVs expressing mFKN or sFKN are efficient means to deliver FKN isoforms to the retina and the brain. Delivery of rAAV-sFKN, compared to rAAV-mFKN, ameliorates activation of microglia, vascular damage and rescues neuronal loss. Therefore, FKN appears to act as a neuroprotective agent in the diabetic retina and potentially serves as an alternative pathway to implement novel translational and therapeutic approaches.

Disclosures: D. Rodriguez: None. **K.A. Church:** None. **A.N. Pietramale:** None. **S.M. Cardona:** None. **D. Vanegas:** None. **K.R. Nash:** None. **A.E. Cardona:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01EY029913
NIH Grant GM061655

Title: Microglia depletion elicits neuroprotective effects to alleviate vascular damage and neuronal cell loss in the diabetic retina

Authors: *K. A. CHURCH[1], D. RODRIGUEZ[2], I. LOPEZ-GUTIERREZ[4], D. VANEGAS[3], S. M. CARDONA[3], A. E. CARDONA[5];
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Abstract: Diabetic retinopathy (DR), an incurable eye disease caused by prolonged high glucose levels in the retina, is a common complication of diabetes mellitus and the leading cause of blindness amongst working age adults. Prolonged high glucose levels damage retinal blood vessels leading to hemorrhages, ischemia and ultimately vision loss. Microglia, the resident immune cells of the central nervous system (CNS), are believed to contribute to the development of DR as they are rapidly activated and respond to transient hyperglycemia, and reset the homeostatic threshold of the retina. As hyperglycemia persists (>4 weeks diabetes), capillary damage occurs resulting in serum proteins and DAMPs from the periphery leaking into the retina perpetuating microgliosis, low-level pro-inflammatory cytokine production and vascular damage. Early (6-8 weeks diabetes) lesions of fibrinogen-activated microglia near blood vessels and the associated vascular damage sustain and increase the release of proinflammatory mediators in the diabetic retina that correlate with neuronal loss. Therefore, we transiently depleted microglia to determine the mechanism by which resident retinal macrophages regulate neuronal and vascular damage in the retina at early stages of diabetes. We hypothesize that transient depletion of $CX3CR1^{CreER}$:$R26^{iDTR}$ microglia during the course of disease when microglia receive the early-stage environmental cues of retinal inflammation due to hyperglycemia will induce neuroprotective cues. Utilizing a genetic model to express diphtheria toxin receptor (DTR) under the tamoxifen (TAM) inducible *CX3CR1*-promoter, microglia become susceptible to the effects of diphtheria toxin (DTx). Microglia were depleted in diabetic and control mice for two weeks from six to eight weeks of diabetes. Vascular and neuronal damage were assessed at ten weeks of diabetes, two weeks after microglia depletion concluded. Our findings revealed that transient depletion of microglia induced neuroprotective cues to upregulate $TUJ1^{+}$ neurons and ameliorate

vascular damage in the diabetic retina. After depletion, repopulating microglia displayed a resting phenotype mirroring those of non-diabetic TAM controls. In conclusion these studies suggest that early transient microglia depletion during the course of disease when microglia receive early-stage activation cues, is neuroprotective by inducing the proliferation of a homeostatic microglia cell population that induce axonal regeneration of $TUJ1^{+}$ neurons and vascular repair.

Disclosures: K.A. Church: None. **D. Rodriguez:** None. **I. Lopez-Gutierrez:** None. **D. Vanegas:** None. **S.M. Cardona:** None. **A.E. Cardona:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01AG068286

Title: Activation of the p38 MAPK pathway is required for amyloid beta-induced neuroinflammatory response in human microglia

Authors: H. LIN, E. D. HAMLETT, A. ERGUL, ***G. Y. WANG**;
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Abstract: Alzheimer's disease (AD), the most common cause of dementia, is characterized by the accumulation of extracellular amyloid-beta peptide (Aβ) plaques and hyperphosphorylated tau-containing intracellular neurofibrillary tangles, along with the progressive loss of memory and cognitive functions. In response to Aβ accumulation, microglia are activated to facilitate the clearance of Aβ aggregates, but unfortunately this process can trigger a neuroinflammatory response that could be detrimental to neurons. Several previous studies have suggested that Aβ-induced neuroinflammation may play a significant role in the pathogenesis of a variety of neurodegenerative disorders, such as AD. Interleukin-6 (IL-6) is a well-characterized pro-inflammatory cytokine and its expression levels were significantly increased in the brains of AD patients. However, the mechanisms by which IL-6 expression levels are up-regulated in the brain tissues of AD patients have not been well elucidated previously. The major goal of this study was to investigate the role of the p38 mitogen-activated protein kinase (p38 MAPK) pathway in Aβ-induced IL-6 production in human microglia. In the present study, we show that Aβ oligomers dose-dependently stimulate IL-6 production in HMC3 human microglial cells. We also show that treatment with Aβ promotes the transcription of IL-6 and tumor necrosis factor α (TNFα) mRNA in human microglia and THP-1 human monocytes. Notably, our data indicate that Aβ selectively induces the activation of the p38 MAPK signaling pathway in human

microglial cells. Furthermore, using immunofluorescence and confocal microscopy, we found that phosphorylated (the active form) p38 MAPK and IL-6 expression levels were markedly increased in microglial cells in the brain tissues of AD patients as compared to the age-matched control subjects. Pharmacological inhibition of p38 MAPK by a set of small molecule inhibitors abrogates Aβ-induced IL-6 secretion. Collectively, these results have demonstrated a critical role for p38 MAPK activation in mediating Aβ-induced production of neuroinflammatory cytokines in human microglia, which suggest that the blockade of the p38 MAPK pathway with selective small molecule inhibitors may be exploited as a novel therapeutic approach to alleviate Aβ-induced neuroinflammation in AD.

Disclosures: H. Lin: None. **E.D. Hamlett:** None. **A. Ergul:** None. **G.Y. Wang:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RO1-AG-051902
P30-NS-045758

Title: Traumatic Brain injury Induced Chronic inflammation and cognitive impairment attenuated by inhibition of stimulator of interferon genes (STING)

Authors: *J. M. PACKER, C. E. BRAY, M. WITZEL, M. OUVIÑA, N. BECKMAN, D. ADEKUNLE-ADEGBITE, J. P. GODBOUT;
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Abstract: Neuropsychiatric complications including depression and cognitive decline develop, persist, and even worsen in the years after traumatic brain injury (TBI), negatively affecting the quality of life and lifespan. Mounting evidence indicates that chronic inflammatory processes linger after TBI, but the mechanism that drives this response is unclear. We previously reported that microglia, innate immune cells in the brain, were the primary drivers of chronic inflammation following diffuse TBI in mice. Moreover, the subacute phase of cortical inflammation 7 days post injury (dpi) was dominated by a robust type 1 interferon (IFN) response. Thus, we hypothesize that increased type I interferon signaling is critical in promoting microglial dysfunction and the subsequent transition from acute inflammation to chronic neuroinflammation after brain injury. In the current study, we targeted the IFN pathway for intervention using knock out mice for the transmembrane protein stimulator of interferon genes (STING) and interferon alpha/beta receptor (IFNAR). In these experiments, adult male wildtype (WT), $STING^{KO}$, and $IFNAR^{KO}$ C57BL/6 mice received a diffuse brain injury and indicators of

neuroinflammation and functional recovery were assessed 7 and 30 dpi. As expected, inflammatory (Cd68, Tlr4) and type 1 interferon associated genes (Tmem173, Irf3, Ifi27) were increased 7 dpi in the cortex of TBI mice compared to controls. These TBI induced increases in interferon associated genes 7 dpi were not attenuated in the global IFNAR^{KO} mice and there was no protection against TBI associated cognitive deficits in these mice. However, reduced IFN signaling profiles subacutely in the TBI-STING^{KO} mice was evident compared to TBI-WT mice and this was paralleled by reduced chronic neuroinflammation (Cd68) at 30 dpi. Chronic deficits in cortical and hippocampal dependent memory as determined by the novel object recognition and location tasks 30 dpi were attenuated in the TBI-STING^{KO} mice. Taken together, reducing type I IFN signaling after TBI through a STING-dependent intervention is effective at reducing chronic inflammation and improving functional recovery post-TBI.

Disclosures: J.M. Packer: None. **C.E. Bray:** None. **M. Witzel:** None. **M. Ouviña:** None. **N. Beckman:** None. **D. Adekunle-Adegbite:** None. **J.P. Godbout:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS097545
NIH Grant NS090934
NIH Grant AG047644
NIH Grant NS085207
HHMI James H. Gilliam Fellowships for Advanced Study

Title: Role of C9orf72 in neuroinflammation and neurodegeneration

Authors: *T. A. MOTA[1], I. LORENZINI[2], S. BELL[1], T. E. MAHAN[3], R. G. SATTLER[2], D. M. HOLTZMAN[3], R. BALOH[1], D. LALL[1];
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Abstract: *C9orf72* hexanucleotide expansions are the most common cause of inherited ALS and frontotemporal dementia (FTD) in European and North American populations. The GGGGCC expansion mutation in the region between exons 1a and 1b leads to both gain of function toxicity (RNA foci and RAN dipeptide repeat proteins) as well as decreased expression of the *C9orf72* transcript (loss of function). *C9orf72* repeat expansions are also found in a subset of other neurodegenerative disorders including late-onset Alzheimer's disease and Parkinson's disease. *C9orf72* is highly expressed in myeloid cells, including resident brain microglia and loss of

C9orf72 has been shown to alter immune response in these cells. To better understand connection between loss of *C9orf72* in microglia and neurodegeneration, we analyzed aged wildtype (WT) and *C9orf72* knockout (*C9orf72-/-)* animals. 12-mnth old *C9orf72-/-* mice showed increased microglial lysosomal accumulations and decreased neuronal dendritic branching than WT mice. We also transcriptionally profiled microglia from young and aged *C9orf72* knockout (*C9orf72-/-)* animals. Unbiased hierarchical clustering and integrative WGCNA analysis of the RNAseq data revealed significantly altered signatures in *C9orf72-/-* microglia, with upregulation of a gene network involving response to aggregates/neuronal injury containing Trem2 and Tyrobp, as well as a network involved in antigen presentation. To identify how altered microglial function from loss of *C9orf72* affects Aβ clearance and neuronal damage, we crossed *C9orf72-/-* animals to the 5XFAD model of Alzheimer's disease (5XFAD:C9orf72-/-) and found that *C9orf72* deficiency dramatically reduced amyloid plaque burden when compared to 5XFAD animals. 5XFAD:C9orf72-/- animals had less astrogliosis, microgliosis and fewer dystrophic neurites. While markers of overall inflammation were decreased, plaques in 5XFAD:C9orf72-/- animals were more dense, less diffuse and were associated with increased numbers of microglia. Interestingly 5XFAD:C9orf72-/- animals showed enhanced loss of dendritic branching than *C9orf72-/-* animals at similar age. Taken together, these data indicate that loss of *C9orf72* leads to intrinsic changes in microglial function that enhances plaque clearance and diminish neuronal pathology in 5XFAD animals although leading to decreased synaptic branching indicative of synaptic loss. This surprising scenario supports that loss of C9orf72 impacts microglial cells in multiple ways that synergistically influences the development of aggregate pathology and synaptic integrity in the brain.

Disclosures: T.A. Mota: None. **I. Lorenzini:** None. **S. Bell:** None. **T.E. Mahan:** None. **R.G. Sattler:** None. **D.M. Holtzman:** None. **R. Baloh:** None. **D. Lall:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: P01 AG055367

Title: Role of microglial TLR4 in obesity-induced neural impairments

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Abstract: Obesity is associated with adverse effects on the brain and neural function, including reduced hippocampal volume, acceleration of brain aging, and increased risks for developing

Alzheimer's disease (AD) and vascular dementia. Obesity is also characterized by chronic and low-grade inflammation that affects numerous tissues. In the brain, obesity drives chronic neuroinflammation in part by increasing microglial activation and production of pro-inflammatory cytokines. Among all pro-inflammatory signaling pathways, toll-like receptor 4 (TLR4) signaling pathways may be particularly important in driving obesity-associated inflammation. TLR4 signaling is implicated as an essential regulator of systemic effects of obesity in metabolic measures and inflammation. Genetic knockout and pharmacologic inhibition of TLR4 can attenuate glucose dysregulation, insulin resistance and inflammation in peripheral tissues. TLR4 signaling in microglia has been suggested to mediate the progression of AD and several other neural disorders but its role in obesity-induced neural outcomes in is largely unknown. Therefore, we generated and analyzed mice that carry a microglial-specific deletion of TLR4 following 16 weeks of exposure to either a control or high fat diet. In male mice, absence of microglial TLR4 had no significant effects on body weight, adiposity, and metabolic measures. However, we observed significant reduction in adipose inflammation in TLR4 knockout mice fed on high fat diet. In females, lacking microglial TLR4 was associated with significant reduction in body weight and improvement of metabolic function. Furthermore, obese mice with microglial TLR4 deletion exhibited significant protection against diet-induced glial activation and impaired neurogenesis both in males and females. These findings implicate an important role for microglial inflammation in obesity-induced neural impairments. Additionally, these findings point to the potential for clinical translation of TLR4 as a therapeutic target for obesity-induced neural injury.

Disclosures: J. Liu: None. **C.J. Pike:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS093416

Title: Microglial morphology and processes stability in a neuro-inflammatory model of cerebral palsy

Authors: *A. CHIME[1], N. SAH[1], A. FOWLER[1], A. SHARMA[2], B. S. SLUSHER[3], R. KANNAN[2], S. KANNAN[1];
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Abstract: Microglia are the resident immune cells of the central nervous system exhibiting heterogeneous and pleomorphic morphology. These microglia continually extend and retract their processes for surveillance, phagocytosis, and chemotactic migration that are dynamically modulated by niche environmental cues including neuronal hyper or hypo-activity. Microglia in the rabbit model of cerebral palsy (CP) expresses a high level of glutamate carboxypeptidase II (GCPII), which facilitates higher extracellular glutamate production and potentially contributes to neuronal hyperexcitability and excitotoxicity. We hypothesized that the dynamics of microglial processes in CP and healthy newborn kits would differ and antagonizing GCPII would influence microglial motility. Using the maternal inflammation-induced fetal-brain injury model of CP in rabbits, we explored the hippocampal microglial motility with confocal live imaging in ex-vivo brain slice preparations. We observed that cell body and phagocytic cup sizes are smaller in healthy controls compared to CP kits and microglial processes are less stable in CP kits, even when microglia are amoeboid in both healthy and CP kits. Treatment of CP kits with dendrimer conjugated 2-PMPA (d-2PMPA), a potent and selective inhibitor of GCPII, induces profound reversal of microglial process dynamics to healthy control levels within 48 hours. Our results emphasize the importance of microglial processes dynamics in determining the state of microglia in the developing brain and demonstrate how acute GCPII inhibition through d-2PMPA can effectively influence microglial motility to healthy control levels.

Disclosures: A. Chime: None. **N. Sah:** None. **A. Fowler:** None. **A. Sharma:** None. **B.S. Slusher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics. **R. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics. **S. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Regulation of ionizing radiation-induced iNOS expression by a novel long non-coding RNA in microglia and a human neuroblastoma cell line

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Abstract: Exposure of the central nervous system to ionizing radiation results in inflammation produced in part by microglial activation. Increased understanding of the regulatory factors

controlling microglial activation and direct neurotoxicity in response to ionizing radiation may aid in preventing inflammation in the CNS. Long non-coding RNAs (lncRNAs) are a class of RNA molecules that regulate gene expression via interactions with RNA-binding proteins, such as transcription factors. While many lncRNAs have been identified, the function of few has been elucidated, and thus lncRNAs represent new potential therapeutic targets to prevent inflammation in the CNS.
Our hypothesis is that the lncRNA Nostrill is induced in response to ionizing radiation and regulates the expression of iNOS, a potent neuroinflammatory mediator. Ionizing radiation was delivered using a Faxitron CellRad irradiator with varying doses of radiation to the BV2 mouse microglial cell line and human SH-SY5Y neuroblastoma cell line. Knockdown was performed using siRNA directed against the gene of interest or a scrambled control siRNA. Overexpression was performed by transfection of an expression vector containing the gene of interest, or an empty vector control.
Nostrill and iNOS both displayed a dose-response relationship, with maximal induction occurring with exposure to 10 Gy ionizing radiation. Time course analysis revealed the most significant induction of both at 6h after exposure. Interestingly, knockdown of Nostrill prior to irradiation significantly reduced the expression of iNOS to levels not significantly different than non-irradiated cells transfected with a scrambled siRNA. Conversely, overexpression of Nostrill prior to irradiation significantly enhanced the expression of iNOS. Overexpression of Nostrill without radiation exposure significantly increased iNOS expression compared to empty vector control. Translational studies in the human SH-SY5Y neuroblastoma cell line demonstrated induction of a positionally conserved lncRNA in response to ionizing radiation.
These preliminary studies demonstrated that the novel lncRNA Nostrill is induced in microglia after exposure to ionizing radiation. Further, based on the results of Nostrill knockdown or overexpression, Nostrill appears to regulate the radiation-induced expression of iNOS. Additionally, a positionally conserved lncRNA is induced in a human neuroblastoma cell line. Further studies will investigate the molecular mechanism of this regulation, and assess biological relevance based on neurotoxicity in co-culture assays.

Disclosures: N.W. Mathy: None. **A. Shibata:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Low dose CSF1R inhibition modulates microglia and macrophage response to disease in the mouse EAE spinal cord

Authors: *D. OFENGEIM, **T. HAMMOND**, F. RAPINO, M. LAMORTE, J. SALEH, J. PROTO, J. KANE, N. HAGAN;
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Abstract: At high doses CSF1R inhibitors rapidly deplete microglia in the mouse brain and have been used to study microglia function in the health and disease. However, at lower concentrations CSF1R inhibition suppresses microglia inflammatory responses in disease (Hagan et al., 2020; Jafari et al., 2021) without depleting microglia. To better understand the effect of low dose CSF1R modulation on microglia and macrophage responses and cell state in the central nervous system we performed single cell RNA sequencing (scRNAseq) on mice with experimental autoimmune encephalitis (EAE), a demyelinating autoimmune disease that triggers infiltration of T-cells and monocytes and causes microglia and astrocyte activation. We isolated immune cells and glia from the adult mouse spinal cord using transcriptional inhibitors to minimize cell isolation artifacts. Animals were treated with a low dose of a novel CSF1R inhibitor (CSF1Ri) following induction of disease. CSF1Ri treatment significantly reduced disease score compared to vehicle treated animals. scRNAseq analysis of the spinal cords uncovered a major shift in the spinal cord cellular milieu including increased numbers of activated microglia and infiltration of dendritic cells, multiple monocyte populations, and T cells. The CSF1Ri had little or no effect on the dendritic cells, astrocytes, and oligodendrocytes/OPCs and modest effects on the T cells transcriptional signature. However, the CSF1Ri significantly shifted the microglia and monocyte cell state, possibly contributing to the reduction in the inflammatory disease response and improved disease score. Microglia and monocytes cell states were also examined by flow cytometry which demonstrated a shift in microglia and monocytes cell subpopulations with CSF1Ri treatment. These results highlight the therapeutic potential of CSF1R inhibition to modulate microglia and macrophage cell state in inflammatory and neurodegenerative disease.

Disclosures: D. Ofengeim: A. Employment/Salary (full or part-time):; Sanofi. **T. Hammond:** A. Employment/Salary (full or part-time):; Sanofi. **F. Rapino:** A. Employment/Salary (full or part-time):; Sanofi. **M. Lamorte:** A. Employment/Salary (full or part-time):; Sanofi. **J. Saleh:** A. Employment/Salary (full or part-time):; Sanofi. **J. Proto:** A. Employment/Salary (full or part-time):; Sanofi. **J. Kane:** A. Employment/Salary (full or part-time):; Sanofi. **N. Hagan:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 MH087332
MH104131
MH105330
DA052209
DA026306 (P5) to M.K.

Title: Microglia specific p38αMAPK signaling is critical in NeuroHIV

Authors: *D. BHULLAR, R. MAUNG, M. KAUL;
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Abstract: HIV-1 causes severe and progressive neurological impairment in humans known as HIV-associated neurocognitive disorders (HAND). The HIV-1 envelope glycoprotein gp120 is an extracellular protein that is shed from infected cells and interacts with distant uninfected brain cells like microglia, neurons and astrocytes. Transgenic mice expressing HIV-1 coat glycoprotein gp120 in astroglial cells of the brain (HIVgp120tg) display neuropathological features similar to HIV dementia patients. During inflammation, p38alpha MAPK is known to regulate the biosynthesis of pro-inflammatory cytokines in endotoxin-stimulated monocytes. Thus, p38 MAPK may be an important mediator in the development of HAND and immunodeficiency during HIV-1 infection. To determine the role of microglial p38 signaling in HIV mediated neuronal injury in vivo, we generated p38 floxed/CX3CR1-Cre HIVgp120-expressing mice that have a p38 knockout specifically in microglia and macrophages. Brain sections of these mice were stained for neuronal MAP2, synaptophysin and GFAP. Astrocytic GFAP levels remained unaffected by microglial p38 deficiency. However, we observed a significant loss of MAP2 and synaptophysin compared to non-tg controls only in HIVgp120tg animals with p38-expressing microglia but not in HIVgp120- expressing brains with microglia-specific p38 knockout, indicating p38 deficiency in microglia protected neurons from HIVgp120 toxicity. Further, we sought to analyze the changes in the microglial transcription profile in HIV glycoprotein expressing mice. We sorted by flow cytometry microglial cells from CX3CR1-Cre positive and negative gp120 tg/floxed p38alpha and non-tg/floxed p38alpha mice for gene expression analysis using RNA seq. Altogether, our study shows in vivo the critical role of microglial p38alpha MAPK in the neurotoxicity caused by the HIV-1 envelope protein, and provided a transcriptional profile of p38alpha-deficient microglia.

Disclosures: D. Bhullar: None. **R. Maung:** None. **M. Kaul:** None.

Poster

P308. Neuroinflammation: Microglia II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P308.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: I01BX004583
W81XWH-19-1-0513

Title: Permethrin Exposure Primes Inflammatory Stress Response in Mouse Model of Gulf War Illness

Authors: *S. X. NAUGHTON[1], U. IQBAL[1], K. TRAGESER[1], H. WU[1], G. M. PASINETTI[1,2]; [1]Icahn Sch. of Med. at Mount Sinai, NewYork, NY; [2]James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: Gulf War Illness (GWI) is a chronic multi-symptom illness affecting roughly one third of military Veterans who served in Operation Desert Storm (1990-1991). Military service members serving in Operation Desert Storm were exposed to numerous toxic chemical agents such as pesticides, chemical weapons, and smoke from oil well fires. Additionally, service members prophylactically consumed the anti-nerve agent pyridostigmine bromide and reported high levels of stress. Soon after, a subset of Veterans began reporting a diverse array of symptoms collectively known as Gulf War Illness (GWI). In particular, Veterans suffering from GWI display a number of cognitive and psychiatric disturbances in addition to other symptoms such as chronic fatigue, gut dysbiosis, and neuropathic pain. Due to the unique combination of exposures the exact cause of GWI is not entirely known, making it difficult to develop accurate preclinical models to evaluate potential therapeutic interventions. The present study seeks to investigate the hypothesis that exposure to the pesticide permethrin primed Veterans to exhibit a heightened response to combat related stressors resulting in long term manifestations of psychiatric illness. Mice were chronically exposed to permethrin or Vehicle for 14 days followed by 7 days of unpredictable stress or gentle handling (control). Mice were then tested for anxiety like behaviors using the open field test and depressive like behaviors using the forced swim test. Our results indicate that exposure to permethrin followed by stress results in depressive, but not anxiety like behaviors. Subsequent histological analysis revealed morphological indications of microglial activation in the hippocampus of mice exposed to both permethrin and stress. We next utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to block microglial priming during permethrin exposure. Transgenic mice which express G_i DREADD receptors on microglia were treated with the DREADD ligand JHU37160 during permethrin exposure, prior to stress. Microglial inhibition via DREADDs was sufficient to block microglial priming by permethrin and blocked the depressive phenotype. These findings shed light on the potential etiology of GWI, particularly with respect to psychiatric and neurological symptoms. Additionally, these results hold important implications for the Gulf War Veteran population who may be more susceptible to lifestyle factors such as stress. The preclinical model developed in this study holds important ramifications for the development of successful therapeutic strategies to treat this underserved Veteran population.

Disclosures: S.X. Naughton: None. **U. Iqbal:** None. **K. Trageser:** None. **H. Wu:** None. **G.M. Pasinetti:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH IAA AOD21005-001 00000

Title: Hypothermia to Recover Neuropathology and Survival after Soman-Induced Seizure

Authors: *C. MUÑOZ, T.-M. SHIH, C. ACON-CHEN, Z.-M. KEITH;
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Abstract: Organophosphorus nerve agents (NAs) irreversibly inhibit acetylcholinesterase in the cholinergic synapses and neuromuscular junction, yielding excitotoxic effects. Current medical countermeasures do not fully protect against NA-induced sustained *status epilepticus* (SSE) and brain damage. Previous animal model work verified that acute treatment with the A1 adenosine (ADO) receptor (A1AR) agonist (±)-5'-Chloro-5'-deoxy-ENBA (ENBA) suppressed SSE with improved neuropathology outcomes. However, severe hypothermia, hypotension, and sedation were experienced post-treatment. Hypothermia alone may shield the brain from injury by slowing down chemical reactions and lessening inflammation which could then recover the neurological outcome after NA-induced SSE. Therefore, we attempted to isolate the hypothermic effect from ADO and assessed the neuroprotective efficacy of direct systemic body cooling in a rat NA SSE model. Results were measured by survival, seizure termination, and neuropathology between ENBA administration and systemic body cooling. Male Sprague Dawley rats were surgically implanted with a body temperature transponder and electroencephalographic electrodes for recording brain activity. A week later, the rats were pretreated with HI-6 intraperitoneally to prevent peripheral cholinergic symptoms and thus promote survival without affecting central activity. Thirty minutes later, rats were exposed subcutaneously to soman or saline (control exposure) and then treated with ENBA intramuscularly or had their body temperature lowered directly via body cooling at 30 min after the onset of seizure. Afterwards, rat groups either were allowed to develop hypothermia as expected or received thermal support to maintain normothermic body temperatures for a period of 6 hours. The surviving rats were euthanized, perfused, and scored for neuropathology 24 hours after exposure. In terms of survival, all hypothermic soman-exposed groups regardless of ENBA or systemic cooling had significantly improved 24-hour survival compared to rats with normothermic temperatures (70.6% vs. 29.4%, p < 0.001). Seizures were equally terminated and neuropathology scores were significantly lower (p<0.01) in soman-exposed rats treated with ENBA, regardless of temperature. Thus, ENBA offered neuroprotection independently of hypothermic body temperatures. Hypothermia enhanced the overall efficacy of ENBA by improving survival outcomes but did not reduce seizure activity or neuropathology after NA-induced SSE.

Disclosures: C. Muñoz: None. **T. Shih:** None. **C. Acon-Chen:** None. **Z. Keith:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant GM112696

Title: Honokiol acts as a neuroprotective in alcohol-induced developmental neurotoxicity in brain organoids.

Authors: *T. ARZUA, Y. YAN, C. JIANG, S. LOGAN, X. BAI;
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Abstract: Alcohol consumption by pregnant women can adversely affect the developing fetus, resulting in a spectrum of deficiencies known as Fetal Alcohol Spectrum Disorders (FASD), with a prevalence of 1.1%-5.0% in the USA. One significant consequence of FASD is alcohol-induced developmental neurotoxicity (AIDN) manifesting as cognitive impairment and behavioral problems throughout life, possibly related to neuronal cell loss, with no effective neuroprotective approach available. Honokiol, a non-pharmaceutical natural compound isolated from Magnolia tree bark, traditionally used in eastern medicine, has been recently shown to act as a protective agent in cerebrovascular injuries, epilepsy, and Alzheimer's disease. We have shown that brain organoids derived from human induced pluripotent stem cells are a promising human-relevant *in vitro* system to study the toxic effects of alcohol. With that, we hypothesized that honokiol could attenuate alcohol-induced toxicity in brain organoids. We found that alcohol concentration-dependently induced neuroapoptosis in organoids as evidenced by the increased activated caspase 3 expression and activity. Pretreatment with 50 μM honokiol for 12 hours significantly attenuated 6-hour alcohol (50 mM) exposure-induced neuroapoptosis. The 50 mM of alcohol concentration is equivalent to a blood alcohol content of 230 mg/dL, mimicking a binge-drinking episode. RT-PCR analysis showed that honokiol upregulated key neuroprotective genes, such as the immediate-early gene NPAS4. Additionally, Seahorse metabolic analysis revealed that honokiol significantly improved mitochondrial function in the alcohol-treated organoids compared with the vehicle control DMSO-treated organoids. Collectively, our findings not only corroborate the versatility of brain organoids as models for AIDN but also the novelty of honokiol as a possible neuroprotective route against alcohol-induced brain injury during development.

Disclosures: T. Arzua: None. **Y. Yan:** None. **C. Jiang:** None. **S. Logan:** None. **X. Bai:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Contemplados Ed 02/2019 - Programa Redes de Pesquisa em Nanotecnologia RJ

Title: The miRNA content of Extracellular Vesicles produced by human mesenchymal stem cells in response to a damaged nervous tissue

Authors: *A. J. DA SILVA, Jr[1,2], P. DOMIZI[4], V. BODART-SANTOS, SR[5], L. R. P. DE CARVALHO[1], R. S. LINDOSO[1], R. MENDEZ-OTERO[6,3];
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Abstract: Our group has been obtaining positive results with cell therapy using mesenchymal stem cells (MSC) to treat injury models in the central nervous system. The therapeutic effect of MSC has been attributed mainly to paracrine mechanisms by releasing a wide variety of free molecules and extracellular vesicles (EVs). EVs are membrane nanoparticles that carry bioactive lipids, proteins and RNA - especially miRNAs, which can modify the physiology of the target cell. We observed that treatment with human-derived MSC (hMSC) promotes neuroprotective and regenerative effects. In this project, the objective is to characterize the EVs generated by hMSCs under different culture conditions in relation miRNA content. The hMSCs were grown under different conditions: in the presence of serum (Control); in the absence of serum for 24 hours (SD); and in the presence of damaged nervous tissue concomitant to the absence of serum (SD+Retina). We observed that most of the miRNAs present in hMSCs remain unchanged in all conditions, which are related to functions common to mesenchymal cells, cell cycle control, oxidative stress and angiogenesis. However, some miRNAs were found exclusively in the Control, SD and SD+Retina conditions, respectively. These molecular changes are reflected in the content of EVs produced by hMSCs under SD and SD+Retina conditions. Even so, EVs of both conditions contain miRNAs associated with the regulation of apoptosis and the modulation of several signaling pathways. Thus, we observed that the conditions of cultivation, especially the presence of damaged nervous tissue, alters the expression profile of miRNAs in hMSCs and their EVs, however several miRNAs associated with their therapeutic potential are not affected by the culture conditions.

Disclosures: A.J. da Silva: A. Employment/Salary (full or part-time):; FAPERJ. **P. Domizi:** None. **V. Bodart-Santos:** None. **L.R.P. de Carvalho:** None. **R.S. Lindoso:** None. **R. Mendez-Otero:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT CIENCIA SIN FRONTERA1783
PAPPIT 214821

Title: Changes in cortical MCP1 levels contribute to higher mortality after brain ischemia in a high-fat diet-induced model of obesity in C57 mice.

Authors: *Y. RODRIGUEZ CORTÉS[1], *Y. RODRIGUEZ CORTES[2], J. RAMIREZ CARRETO[3], L. CONCEPCIÓN CARRILLO[4], E. LOPEZ HERNANDEZ[5], H. LOPEZ VALDEZ[3], A. CHAVARRIA KRAUSER[3];
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Abstract: Obesity is a chronic low-grade systemic inflammatory statethat is the sixth leading cause of death worldwide. Obesity gradually activatesthe immune system inducing a constant release of pro-inflammatory cytokines inlow amounts that are produced in part by the visceral adipose system, leadingto the activation of pathways of inflammation, and contributing to thedevelopment of serious complications such as insulin resistance.Obesity-associated systemic inflammation causes increased blood-brain barrierpermeability, glial activation, and secondary neuroinflammation which is a riskfactor for developing other brain pathologies Among the brain structuresaffected with neuroinflammation associated with obesity are the cortex,hippocampus, brain stem, among others. Since neuroinflammation secondary toobesity is chronic, we wanted to see how this neuroinflammation may impact anacute event such as brain ischemia. Male C57/Bl6 mice were fed with a high-fatdiet (for 12 weeks a Bodyweight was monitored weekly, fasting glucose, andintraperitoneal glucose were determined after 12 weeks of diet and were thensummited to cortical photothrombotic to induce brain ischemia TNF α IL10, MCP1,and BDNF levels were measured by ELISA before ischemia in the cortex andstriatum. Mice with HFD gained significantly more weight than subjects fed withnormal diet from the third week of diet and glucose metabolism was also alteredsince fasting glucose and area under the curve of the glucose tolerance testwere significantly higher in HFD mice. HFD mice presented higher corticallevels of MCP1 and BDNF compared to control mice. The other cytokines did notpresent any difference when compared to the control group neither in the cortexnor the striatum. We also

found differences between the cortex and the striatumin the levels of fractalkine in the HFD group and MCP1 in the control group. Inthe other cytokines, we did not find significant differences in the brain areasanalyzed. HFD mice with ischemia presented 50% mortality compared to 100%survival of the control group. Higher cortical levels of MCP1 correlate s withperipheral findings in adipose and liver tissue of obese animals and may be aresult of the inflammatory function of microglia
Funding: CONACYT Ciencia deFrontera 2019(1783) and Universidad NacionalAutónoma de México PAPPIIT (IN214821).

Disclosures: Y. Rodriguez cortes: None. **J. Ramirez carreto:** None. **L. Concepción carrillo:** None. **E. Lopez hernandez:** None. **H. Lopez valdez:** None. **A. Chavarria krauser:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council (NSERC)

Title: Lithium induced neuroprotection is mediated by mesencephalic astrocyte-derived neurotrophic factor (MANF)

Authors: *F. A. ABU-HIJLEH[1], V. PATEL[1], N. RIGG[1], B. N. FREY[1,2], R. K. MISHRA[1]; [1]Psychiatry and Behavioural Neurosci., McMaster Univ., Hamilton, ON, Canada; [2]Mood Disorders Program and Women's Hlth. Concerns Clin., St. Joseph's Healthcare, Hamilton, ON, Canada

Abstract: Background: Although introduced 70 years ago, the monovalent cation lithium remains the 'gold standard' treatment for bipolar disorder (BD); however, its therapeutic mechanism of action remains elusive. Accumulating data suggest that lithium's neuroprotective properties involve the modulation of ER-calcium binding proteins such as GRP78, coined the 'master regulator' of endoplasmic reticulum (ER) stress. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER-resident protein with demonstrated neuroprotective capabilities against cellular insults through its direct interaction with GRP78. The purpose of this study was to determine whether lithium neuroprotection against ER stress is associated with an increase in MANF and GRP78 through the AP-1 pathway. Additionally, ER-stress-associated proteins such as Bcl-2 and CHOP were investigated. **Methods:** Mouse striatal cells were pretreated with either PBS, lithium or lithium + AP-1 inhibitor for 6 days before being exposed to thapsigargin, an inducer of cytotoxic ER-stress for 24 hours. Cell viability gene and protein expression were quantified using MTT assays, RT-qPCR and western blots, respectively.

Results: (1) Protracted lithium treatment provides neuroprotection against thapsigargin-induced cell death. (2) Lithium neuroprotection was correlated with the elevated expression of MANF alongside pro-survival ER-stress-associated proteins, GRP78 and Bcl-2 (3) Lithium neuroprotection was attenuated upon the addition of a potent AP-1 inhibitor, alongside the diminished increase in expression of MANF and GRP78. **Conclusions:** Taken together, these data suggest that lithium's neuroprotective mechanisms involve the modulation of pro-survival ER-resident proteins such as MANF through the AP-1 transcription factor. Knockout studies using the CRISPR-Cas9 system will determine the extent of MANF's involvement in lithium neuroprotection. These findings warrant further investigation of MANF as a novel therapeutic for BD.

Disclosures: F.A. Abu-Hijleh: None. **V. Patel:** None. **N. Rigg:** None. **B.N. Frey:** None. **R.K. Mishra:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Selenium mitigates prenatal lead-induced toxicity on cerebral cortex of Wistar rat pups

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Abstract: Lead (Pb) is a dangerous substance to the body that particularly targets the central nervous system (CNS), especially during the early stages of development. Unfortunately, classical therapies remain inefficient in mitigating neurotoxicity associated with developmental Pb exposure on brain regions. However, co-occurring selenium, and heavy metals are known to reduce each other's effects. In this study, the counteractive impact of selenium (Se) to the toxic effects of Pb on the developing rat brain was investigated to explore early protection against developmental disruption of the cerebral cortex by Pb, using Wistar rat model. Pregnant rats were grouped into 3 group of 3 animals each. Group 1 served as control and received 2ml distilled water, group 2 received 60 mg/kg of Pb and group 3 received 60 mg/kg of Pb and 0.3 mg/kg of Se. Administration was orally from gestation day 9 till parturition. On PND 1, and 21, brain tissues were harvested for biochemical and histopathological (histochemical and histological) studies. Observations show that lead accumulated in the brain of pups in an inverse relationship with calcium. Pups in the group administered only Pb, showed evidence of serious necrosis, and neuronal degeneration when compared with the control group and the group co-administered Se and Pb. Hence, the gestational neurotoxic effect of Lead on the cerebral cortex

can be mitigated by Se. Our findings show that Selenium, an essential trace mineral of fundamental importance for animals and humans, might be beneficial in lead toxicity therapy.

Disclosures: B.E. Echefu: None. **S.A. Musa:** None. **U.E. Umana:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1R01 NS114578-01 (EJB)
NIH Grant T32HD094671 (ASC)
Jean and George Brumley Jr. Neonatal Perinatal Research Institute
AAP Marshall Klaus Neonatal-Perinatal Research Award (ASC)

Title: Breast milk-associated oxysterol reverses neonatal white matter injury through Gli2-dependent oligodendrogenesis

Authors: *A. S. CHAO[1], P. MATAK[1], K. PEGRAM[1], L. DUBOIS[2], J. W. THOMPSON[2], V. JAIN[3], N. YOUNGE[1], S. GREGORY[3], R. N. GOLDBERG[1], E. J. BENNER[1];
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Abstract: White matter injury (WMI) is the most common brain injury leading to poor neurologic outcomes in premature infants. Sepsis and inflammation are significant risk factors and there are no treatment options available. Developing novel drug therapies for neonates is challenged by appropriate concerns for safety. Here, we identified oxysterols in human maternal breast milk and explored their therapeutic potential in directing neural stem cells (NSCs) into the oligodendrocyte (OL) lineage via the Sonic HedgeHog (Shh) pathway *in vitro*. Further, we investigated whether breast milk-associated oxysterols can rescue neonatal WMI *in vivo*. Oxysterols (20HC, 22(S)HC, 22(R)HC, 24HC, 25HC, 27HC) were identified in breast milk using Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry. NSCs were cultured from the subventricular zone (SVZ) of mice and treated with oxysterols. Cells were analyzed by immunohistochemistry, western blot, flow cytometry, and 10X single cell droplet sequencing, looking for markers of the OL lineage. Shh pathway activation was established by quantifying upregulation of target gene, Gli1, by western blot and RT-PCR. Gli-dependence was explored using pharmacological and genetic approaches. Neonatal sepsis leading to WMI was induced in mice on postnatal day 5. Mice then received either 20HC or vehicle. Stereology determined OL numbers in the periventricular white matter regions. Myelination was evaluated

by performing g-ratios to determine myelin thickness and using an unbiased Space Balls stereological probe to quantify subcortical myelinated fiber length. Motor function in mice was analyzed using the CatWalk gait analysis system. To lineage trace postnatal SVZ NSCs *in vivo*, neonatal sepsis was induced in Nestin-CreER^{T2};R26r-TdTomato pups as above, and then treated with either vehicle or 20HC. We found that Gli2 is functionally required for 20HC-induced oligodendrogenesis. Following neonatal WMI *in vivo*, 20HC treatment increased numbers of mature OLs, improved myelination and rescued motor deficits in mice. Lineage tracing experiments showed that 20HC-mediated recovery of OL deficit is mediated in part through 20HC-induced SVZ-derived oligodendrogenesis *in vivo*. Because oxysterols are found in human maternal breast milk, this approach may be further developed into a novel and safe therapeutic strategy to mitigate neonatal WMI.

Disclosures: A.S. Chao: None. **P. Matak:** None. **K. Pegram:** None. **L. DuBois:** None. **J.W. Thompson:** None. **V. Jain:** None. **N. Younge:** None. **S. Gregory:** None. **R.N. Goldberg:** None. **E.J. Benner:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS110648
Astrazeneca supplied saracatinib (Open Innovation Program)

Title: Saracatinib, a src family kinase inhibitor, mitigates gliosis and neurodegeneration in the rat (diisopropylflourophosphate) DFP model

Authors: *M. C. GAGE[1], L. WACHTER[4], K. DISHMAN[4], M. R. PUTRA[2], T. THIPPESWAMY[3];
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Abstract: The present study aims to determine the disease modifying potential of saracatinib, a src family kinase inhibitor, in diisopropylfluorophosphate (DFP) induced toxicity. DFP and other organophosphate nerve agents initiate *status epilepticus* (SE) via the irreversible inhibition of acetylcholinesterase. Src family kinases are upregulated following SE and are implicated in both neuroinflammatory pathways (leading to the production of proinflammatory cytokines and reactive oxygen species) and neuronal pathways associated with hyperexcitability. We therefore predicted that saracatinib treatment after SE could mitigate DFP-induced neuropathology and brain dysfunction. Male Sprague Dawley rats (7-8 weeks) were used in this study. The rats were

exposed to 4 mg/kg DFP or PBS (subcutaneous) followed immediately by 2 mg/kg atropine sulfate (intramuscular) and 25 mg/kg 2-pralidoxime (intramuscular). Midazolam (3 mg/kg, intramuscular) was administered after 20 minutes of convulsive seizures (around 30 minutes after DFP) to terminate behavioral seizures. Two hours after midazolam, saracatinib (20 mg/kg, o.p.) or vehicle was given once a day for seven days. These animals were assessed 6 weeks later for behavioral comorbidities and then terminated at 10 weeks to assess gliosis and neurodegeneration via immunohistochemistry. We found nonsignificant reduction in discrimination indexes in the novel object recognition test in DFP treated animals with minimal mitigation by saracatinib. DFP treated animals, both those administered vehicle and saracatinib, reached the goal box quicker than controls in the horizontal bar test. There were no differences between groups in the Morris Water Maze, forced swim test or rotarod apparatus. At 10 weeks, DFP significantly increased microgliosis, astrogliosis and neurodegeneration; this was significantly mitigated by saracatinib in the piriform cortex and amygdala. These findings suggest saracatinib could be a useful therapeutic intervention in the mitigation of brain pathology following organophosphate toxicity by DFP. However, the degree of mitigation of behavioral dysfunction by saracatinib require further investigation

Disclosures: M.C. Gage: None. **L. Wachter:** None. **K. Dishman:** None. **M.R. Putra:** None. **T. Thippeswamy:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IBRO Early Career Awards 2020
FAPEMIG - Universal grant (APQ-00218-17)
CNPq - Universal grant (425669/2018-1)
International Society for Neurochemistry (ISN) - CAEN CATEGORY 1B - 2021

Title: Overexpression of miR-17 promotes neuroprotection and regeneration in the central nervous system.

Authors: *F. S. L. CAMBRAIA[1], R. A. D. ALMEIDA[1], C. G. FERREIRA[1], C. R. D. O. CYRINO[1], V. MATOS[1], L. CALDI-GOMES[2], U. MITCHEL[3], V. D. T. RIBAS[1];
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Abstract: Neurons of the central nervous system (CNS) of adult mammals have limited regenerative capacity, with no effective spontaneous regeneration after axonal injury. Consequently, any damage to this system, whether due to neurodegenerative diseases or traumatic injuries, leads to permanent neurological deficits. For example, in glaucoma, a multifactorial neurodegenerative disorder, optic nerve damage leads to irreversible blindness due to the progressive degeneration of the axons that make up the optic nerve and the subsequent loss of retinal ganglion cells (RGCs). Therefore, it is imperative to develop new therapeutic strategies aiming to protect neurons from death, as well as their axons from degeneration, and to promote the regeneration of injured axons. Recently, microRNAs, which are small non-coding RNA molecules involved in the control of gene expression post-transcriptionally, have emerged as interesting targets to promote neuroprotection and regeneration in the CNS. However, little is known about the role of specific microRNAs in CNS repair. In this study, we evaluated the role of miR-17 in neuroprotection and regeneration using *in vitro* and *in vivo* models of CNS injury. For that, recombinant adeno-associated virus (rAAV) vectors overexpressing miR-17 (rAAV.miR-17) or expressing only mCherry protein (rAAV.CTRL) were used to transduce primary cortical neurons *in vitro*and RGCs of adult Wistar rats *in vivo*. In the *in vitro* analysis, primary cortical neuron culture was subjected to a scratch lesion and neuritic regeneration were evaluated. The rAAV.miR-17 was able to significantly increase neurite length. For the *in vivo* analysis, RGCs were transduced with rAAV vectors, retrogradely traced by FluoroGold and subsequently an optic nerve crush was performed. Neuroprotection of RGCs was observed by overexpression of miR-17, with a significant increase in RGCs survival 28 days after the lesion. Axonal staining by the expression of mCherry revealed axonal protection in the optic nerve in the proximal and distal regions to the lesion. Immunostaining for GFAP and Iba1 in optic nerve cryosections showed no change in astrocytes and microglia, respectively. Bioinformatics analysis allowed the identification of predicted and validated mRNA targets of miR-17. Functional analysis of gene ontology of the predicted mRNA targets of miR-17 showed that several of them are involved with regeneration, degeneration and cell death. In conclusion, these findings provide new knowledge about the role of miR-17 in neuroprotection and regeneration in the CNS, and point to miR-17 as a potential therapeutic target for CNS damage, especially those affecting the optic nerve.

Disclosures: F.S.L. Cambraia: None. **R.A.D. Almeida:** None. **C.G. Ferreira:** None. **C.R.D.O. Cyrino:** None. **V. Matos:** None. **L. Caldi-Gomes:** None. **U. Mitchel:** None. **V.D.T. Ribas:** None.

Poster

P354. Neuroprotective Mechanisms: Preclinical: Small Model Therapeutics

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P354.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS Grant 1U01NS102135-01A1

Title: Superior antiseizure and neuroprotective efficacy of LY293558-Caramiphen in combination when compared to midazolam against soman induced seizures in 7 days old rats

Authors: *J. APLAND[1], T. H. FIGUEIREDO[2], M. D. FURTADO[3], V. ARONIADOU-ANDERJASKA[2], V. PIDOPLICHKO[2], K. ROSSETTI[2], M. BRAGA[2];
[1]USAMRICD, Gunpowder, MD; [2]Anatomy, Physiol. & Genet., Uniformed Services Univ., Bethesda, MD; [3]Anatomy, Physiol. and Genet., USU, Bethesda, MD

Abstract: The currently approved anticonvulsant for the treatment of status epilepticus (SE) induced by nerve agents is the benzodiazepine midazolam. However, midazolam does not appear to offer neuroprotective benefits, particularly in children, probably because in the developing brain, synaptic transmission mediated via GABAA receptors, the target of benzodiazepines, is still weak or depolarizing. In the present study, we exposed 7-day-old (P7) male and female rats to 1.2xLD50 soman, and compared the antiseizure, anti-lethality, and neuroprotective efficacy of midazolam (3 mg/kg) and the combination of LY293558 (an AMPA/GluK1 receptor antagonist; 5 mg/kg) and caramiphen (an antimuscarinic with NMDA receptor-antagonistic properties; 50 mg/kg) administered 1 h post-exposure. Treatment with LY293558+CRM showed superior efficacy to stop seizures in P7 rats, the latency for re-occurrence of seizures after cessation of the initial SE was dramatically longer in the rats treated with LY293558+CRM, and the total duration of SE was significantly shorter in this group. The difference between the two treatment groups in the total duration of SE was accompanied by differences in neuropathology. Total neuronal loss and loss of interneurons in the CA1 hippocampal area and the BLA were significant in the MDZ-treated group in comparison with control non-exposed rats and with the LY293558+CRM-treated rats, at 1, 3 and 6 months post-exposure. There is an apparent deterioration over time, and at the 6-month time point, there is also some neuronal and interneuronal loss in the basolateral amygdala (BLA) of the LY293558+CRM treated group. Hippocampal volume was significantly reduced in the MDZ-treated rats but not in the LY293558+CRM-treated rats, at 3 and 6 months post-exposure. Spontaneous, background inhibitory activity was significantly reduced in the MDZ-treated group but not in the LY293558+CRM group, at 1, 3 and 6 months post-exposure, and this was accompanied by increased anxiety only in the MDZ-treated group, as determined by the Open Field and the Acoustic Startle Response tests. The appearance/incidence of spontaneous recurrent seizures was greater in the MDZ-treated group, and the difference from the LY293558+CRM group reached statistical significance at the 3-month time point. The combined administration of LY293558 and caramiphen, by blocking mainly AMPA, GluK1, and NMDA receptors, is a very effective anticonvulsant and neuroprotective therapy against soman in immature rats.

Disclosures: J. Apland: None. **T.H. Figueiredo:** None. **M.D. Furtado:** None. **V. Aroniadou-anderjaska:** None. **V. Pidoplichko:** None. **K. Rossetti:** None. **M. Braga:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.01

Topic: D.03. Somatosensation – Pain

Support: NS042595; The Dr. Seymour and Rose T. Brown Professorship in Anesthesiology
K00NS113422
American Neuromuscular Foundation Career Development Award
F32DA051160-01
American Heart Association - Predoctoral Fellowship

Title: Human Sensory Tissue Extraction and Research: How to Develop a Collaboration with Local Organ Procurement Organizations

Authors: *Z. BERTELS[1], A. CHAMESSIAN[1], D. BROCKMEIER[2], B. A. COPITS[1], J. S. DEL ROSARIO[1], J. LEMEN[2], J.-N. LI[1], G. MARKLIN[2], J. SACKEY[1], R. SLIVICKI[1], A. WIDMAN[1], J. YI[1], R. W. GEREAU, IV[3];
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Abstract: Chronic pain conditions afflict over 100 million individuals in America and cause greatly diminished quality of life. Much is still unknown about the underlying pathophysiology of chronic pain states resulting in unsuccessful treatments and frustration from both patients and clinicians. A major hurdle slowing the progress in the development of new effective treatments is the translation from preclinical data to clinically relevant findings. Investigations of rodent sensory neurons have previously been used to explore molecular mechanisms that contribute to the development of chronic pain states, but these studies can potentially run into late-stage translational issues. The use of human tissues provides a route for direct validation of preclinical findings and further development of clinically relevant assays. Unfortunately, gaining access to human sensory tissue can be difficult and expensive. Collaboration with local organ procurement organizations offers a feasible route for access to human tissues for translational research programs. Here we describe how our group established a relationship with a local organization, Mid-America Transplant, to facilitate this work in our Pain Center. This collaboration has allowed the collection of human dorsal root ganglia, spinal cord, intervertebral discs, sympathetic ganglia and other tissues from an array of donors allowing for a diverse data set. Because of the proximity to the tissue source and optimized protocols, we obtain high quality tissues, allowing for a diverse array of physiologic, genetic and anatomical studies that have proved challenging previously. This allows for direct probing of therapeutic targets or development of new clinically relevant models. This presentation we will provide an outline to the general workflow of obtaining the human sensory tissue and its processing. With the methods outlined in this presentation we hope to discuss replication of this relationship in other labs and further facilitate clinically relevant research.

Disclosures: Z. Bertels: None. **A. Chamessian:** None. **D. Brockmeier:** None. **B.A. Copits:** None. **J.S. Del Rosario:** None. **J. Lemen:** None. **J. Li:** None. **G. Marklin:** None. **J. Sackey:** None. **R. Slivicki:** None. **A. Widman:** None. **J. Yi:** None. **R.W. Gereau:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS111929

Title: Using human nervous tissues from organ donors for therapeutic discovery and translational validation - a collaboration with the Southwest Transplant Alliance and the Center for Advanced Pain Studies at the University of Texas at Dallas

Authors: *S. I. SHIERS[1], J. M. MWIRIGI[1], M. S. YOUSUF[1], D. T. FERREIRA[1], A. CERVANTES[2], J. C. REESE[2], T. J. PRICE[1];
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Abstract: Rodent models have been the standard for preclinical research for decades as they offer an invaluable means to explore the functional, morphological, and molecular properties of disease. However, differences in the underlying biology between rodents and humans exist which most certainly underlies the limited success of rodent-derived therapeutic strategies into the clinic. As such, the Southwest Transplant Alliance and the Center for Advanced Pain Studies at the University of Texas at Dallas have unified forces to tackle this obstacle in therapeutic translatability and discovery. With access to nearly 300 donor collections yearly, we have generated a large tissue bank of fresh frozen human nervous tissue including dorsal root ganglia, spinal cord, vagus nerve, sciatic nerve, spinal meninges, and nodose ganglia. Additionally, with direct access to the living tissues, we can keep these tissues alive as explants for several hours and as cultures for at least 10 days. These tissues are high quality (recovered within 4 hours post-mortem) and can be used for many types of molecular assays. For instance, over the last year, we have identified human-specific analgesic targets, compared the expression of rodent-identified genes and proteins, evaluated morphological and anatomical differences between species, and assessed the functional and molecular changes of human neurons after direct application of a stimulus or therapeutic. Herein, we will discuss how our collaboration works, how we recover human nervous tissues and the basic methods and applications of these tissues for therapeutic discovery. This knowledge can be used by other laboratories and transplant organizations to achieve similar efforts around the world.

Disclosures: S.I. Shiers: None. **J.M. Mwirigi:** None. **M.S. Yousuf:** None. **D.T. Ferreira:** None. **A. Cervantes:** None. **J.C. Reese:** None. **T.J. Price:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.03

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01NS065926-11

Title: Oncostatin M induces MNK-eIF4E signaling in human dorsal root ganglia

Authors: J. MWIRIGI, I. SANKARANARAYANAN, S. SHIERS, M. KUME, P. RAY, M. YOUSUF, T. J. PRICE;
Univ. of Texas at Dallas, Richardson, TX

Abstract: Neuroinflammation drives chronic pain states by modulating protein synthesis, particularly in 5' cap-dependent translation and phosphorylation of the eukaryotic initiation factor 4E (p-eIF4E). As such, oncostatin M (OSM) is part of the interleukin-6 (IL6) family of cytokines and has been implicated in neuropathic pain. However, the precise mechanism by which OSM mediates pain hypersensitivity remains to be elucidated. We have previously reported an increase in OSM transcripts in human dorsal root ganglia (hDRG) obtained from patients suffering from radicular/neuropathic pain. In a separate study using spatial transcriptomics, we show that OSM receptors (OSMR) are predominantly expressed by putative nociceptors. We first validated the effects of OSM on hDRGs by treating acutely sliced explants with 10ng/ml OSM and observed an increase in eIF4E phosphorylation, particularly in small diameter neurons and satellite glial cells. Similarly, treating dissociated human sensory neurons with OSM upregulated p-eIF4E levels in putative nociceptors. Based on this data, we aim to elucidate its role in translation signaling pathways via widescale phospho-proteomic profiling in human DRG neurons. Taken together, our work highlights the potential for new pain therapeutics by targeting inflammatory modulators such as OSM.

Disclosures: J. Mwirigi: None. **I. Sankaranarayanan:** None. **S. Shiers:** None. **M. Kume:** None. **P. Ray:** None. **M. Yousuf:** None. **T.J. Price:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.04

Topic: D.03. Somatosensation – Pain

Support: NS111929
NS065926

Title: Molecular characterization of human dorsal root ganglion (DRG) neurons by spatial RNA sequencing

Authors: *D. TAVARES FERREIRA[1], S. SHIERS[1], P. RAY[1], I. SANKARANARAYANAN[1], M. KOTAMARTI[1], A. CERVANTES[2], J. REESE[2], M. BURTON[1], G. DUSSOR[1], T. PRICE[1]; [1]Univ. of Texas At Dallas, Richardson, TX; [2]Southwest Transplant Alliance, Dallas, TX

Abstract: DRG neurons are responsible for transmitting all somatosensation (touch, proprioception, nociception and temperature) from the body to the spinal cord and brainstem. Transcriptomic studies at single-cell resolution on mouse DRG neurons have contributed to our understanding of pain and sensory mechanisms. However, a detailed molecular characterization of human DRG neurons is missing. To fill this gap in knowledge, we have conducted spatial sequencing experiments on human lumbar DRGs collected from 8 organ donors (two sections per donor). The tissues were sectioned into the capture areas of the Visium slides (containing 55 µm barcoded-spots), stained and imaged. During tissue permeabilization, mRNA from each section bound to barcoded primers and was subsequently processed for library preparation and RNA sequencing. Because each section was stained and imaged, the barcoded mRNAs and respective genes' location can be visualized within each DRG section using Loupe Browser (10x Genomics). To generate near single-neuron resolution, we selected all barcodes that overlapped a single neuron in all sections and processed them for downstream analysis. Using ~4000 barcodes that overlapped a single neuron, we identified 12 subsets of human sensory neurons: one proprioceptor, two Aß low-threshold mechanoreceptors (LTMRs), one Aß nociceptor, one Aδ-LTMR, one Aδ high-threshold mechanoreceptor (HTMR), one C-LTMR and five nociceptor subtypes. We have compared our results to both mouse and non-human primate datasets, finding many similarities, but also important differences. These comparisons highlight important implications for the translation of pain animal models and for pain target identification. We also identified sex differences in human DRG subpopulation transcriptomes, including a significant increase in CALCA expression in female pruritogen receptor-enriched nociceptors. In addition to generating near single neuron resolution, we also looked at the presence of non-neuronal cells in human DRGs. Immune cells such as T-cells and macrophages were detected throughout the DRG tissue, including near neurons. These immune cells express several ligands that can interact with neurons to potentially influence sensory mechanisms. We anticipate that our data will advance our understanding of molecular pain mechanisms in humans and facilitate the translation of basic pain neuroscience research.

Disclosures: D. Tavares Ferreira: None. **S. Shiers:** None. **P. Ray:** None. **I. Sankaranarayanan:** None. **M. Kotamarti:** None. **A. Cervantes:** None. **J. Reese:** None. **M. Burton:** None. **G. Dussor:** None. **T. Price:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.05

Topic: D.03. Somatosensation – Pain

Support: NS111929 (to PMD and TJP)
NS102161 (to TJP)

Title: Transcriptional signatures of pain-associated human DRGs: identifying relevant neuro-immune protein interaction networks from sex differential neuronal and immune gene expression profiles

Authors: *P. RAY[1], S. SHIERS[1], M. L. UHELSKI[2], G. O. DUSSOR[1], P. M. DOUGHERTY[2], T. J. PRICE[1];
[1]Ctr. for Advanced Pain Studies, Behavioral and Brain Sci., Univ. of Texas At Dallas, Richardson, TX; [2]Pain Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Title: Transcriptional signatures of pain-associated human DRGs: identifying relevant neuro-immune protein interaction networks from sex differential neuronal and immune gene expression profiles Authors: Pradipta R. Ray[1], Stephanie Shiers [1], Megan L. Uhelski[2], Gregory Dussor[1], Patrick M. Dougherty[2], Theodore J. Price[1] Affiliations: 1 University of Texas at Dallas, Department of Neuroscience and Center for Advanced Pain Studies; Richardson, TX, USA. 2 MD Anderson Cancer Center, Houston, TX Goals: Over 50 million Americans suffer from chronic pain, with opioids being the primary drug used to treat pain. In order to develop non-opioid drugs for treating chronic pain, molecular mechanisms of chronic pain need to be understood and putative drug targets identified. Our goal is to quantify whole transcriptome RNA abundances using RNA-seq in pain-associated human DRGs from vertebrectomy patients, and to comprehensively identify molecular changes in these samples by contrasting them with non-pain associated DRGs. Methods: RNA was extracted from the excised DRGs, and cDNA library prepared using stranded, single-end library preparation kit from Illumina, mapped to the reference transcriptome using STAR, and relative abundances quantified using Stringtie. Comparing gene expression distributions across pain-associated and non-pain-associated DRGs in a sex-specific manner identify pain-associated genes for males and females. Gene co-expression modules for these differentially expressed genes are identified. Ligand - receptor signaling based on the set of differentially expressed genes reveal a clear picture of sex differential neuro-immune signaling. Results: We have currently sequenced over 80 human

DRGs, with over 50 having mRNA libraries enriched in neuronal mRNA. Our differential expression analysis revealed a sex differential set of differentially expressed genes in males (including IL1B, TNF, CXCL14, OSM) and females (including CCL1, CCL21, IL2RA, PENK and TRPA1). A small subset of pain-associated genes are common to both sexes (GLRA3, BCL3). Co-expression modules reveal enrichment in JUN-FOS signaling in males, and centromere protein coding genes in females. Neuro-immune signalling pathways reveal multiple cytokine signaling pathways in males (OSM, LIF, SOCS1) and females (CCL21, IL2RA). We have validated a some of these findings using RNAscope studies. Conclusions : Sex differential molecular pathology for chronic pain in males and females suggest the need to sex specific pain therapies. Identification of distinct T cell, B cell and macrophage signature reveals the need for immune cell profiling in chronic pain associated tissues.

Disclosures: P. Ray: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder, Doloromics (no role in study). **S. Shiers:** None. **M.L. Uhelski:** None. **G.O. Dussor:** None. **P.M. Dougherty:** 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.; PI : NS111929 (NIH). **T.J. Price:** 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.; PI : NS111929 (NIH), NS102161 (NIH). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder, Doloromics (no role in study).

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.06

Topic: D.03. Somatosensation – Pain

Support: 5R01NS065926

Title: Fmrp is present in the peripheral and spinal arbors of a subset of primary nociceptors in humans.

Authors: *M. E. MITCHELL, D. F. TAVARES, S. I. SHIERS, A. AHMAD, T. J. PRICE; Neurosci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Current chronic pain therapies remain largely ineffective forcing millions of individuals across the globe to overcome their often, persistent and debilitating symptoms without adequate relief. Moreover, advancement in effective therapies is rare. A major obstacle

is that treatments targeting nociceptive mechanisms identified in rodent models rarely perform in clinical settings. This underscores the variation in chronic pain mechanisms between rodents and humans. Therefore, we need to interrogate the basic biology of human nociceptive function and regulation. A wealth of work in rodent models has shown that long-term, maladaptive plasticity in primary nociceptors drives chronic pain states. Activity-dependent changes in axonal translation regulation critically contribute to this. This is best studied in the peripheral branches of primary nociceptors, and emerging evidence indicates local translation also plays a role in their spinal terminals. In humans, it is unclear how local translation regulation in these presynaptic compartments contributes to primary nociceptor plasticity. We approached this question immunostaining and imaging human spinal cord and nerves for Fragile X Mental Retardation Protein or FMRP. It is an RNA-binding protein and critical regulator of local translation in neurons. Mutations in the gene encoding FMRP cause Fragile X Syndrome (FXS), the most common heritable form of autism spectrum disorders (ASD). As in many forms of ASD, FXS is associated with sensory processing deficits, including abnormalities in nociceptive sensitization, that arise from aberrant FMRP-dependent synaptic plasticity. In brain, it is well-established that FMRP couples synaptic signals to changes in local translation. In FXS, this translation is uncoupled from receptor and/or kinase activation impairing synaptic sensitivity and plasticity. Fragile X mice exhibit strikingly decreased nociceptive sensitization, blocked inflammation-induced hypersensitivity, and delayed onset of allodynia in neuropathic pain. This is associated with mTOR signaling that is uncoupled from stimulation in primary nociceptors. In WT mice, FMRP is present in the peripheral and spinal branches of a subset of these afferents where it is in RNPs. Here, we show that FMRP is also present across these branches of a subset of primary nociceptors in human nerve and spinal cord. We found it is notably abundant in distinct primary nociceptive compartments in Lissauer's tracts and in Lamina I/II glomeruli. Overall, these findings are consistent with rodent data indicating that intact sensitization and normal onset of chronic pain require FMRP-regulated axonal translation.

Disclosures: M.E. Mitchell: None. **D.F. Tavares:** None. **S.I. Shiers:** None. **A. Ahmad:** None. **T.J. Price:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01NS065926-11

Title: The integrated stress response and eIF2A regulate methylglyoxal-induced pain hypersensitivity

Authors: *M. YOUSUF[1], J. M. MWIRIGI[7], C. D. GARCIA[2], M. R. PARKS[2], S. SHIERS[3], A. A. KOMAR[8], Z. CAMPBELL[4], G. DUSSOR[5], T. J. PRICE[6];
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Abstract: Diabetes is a metabolic disorder characterized by abnormally high glucose levels due to a lack of insulin production or an insensitivity to insulin. Over 10% of the US population is diagnosed with diabetes and another 35% suffer from prediabetes. As much as half of the diabetic population will develop peripheral neuropathy during their disease course. Previous studies in animals and humans have linked methylglyoxal (MGO), a reactive product of glycolysis, to diabetic neuropathic pain (DNP). However, exactly how MGO causes neuropathic pain in diabetes remains enigmatic. We have recently demonstrated in rodent dorsal root ganglia (DRG) that MGO engages the integrated stress response (ISR) by inhibiting translation elongation through phosphorylating eukaryotic initiation factor 2α (p-eIF2α) via a host of kinases that respond to cellular stress. This results in a repression of global translation while increasing the translation of stress-responsive genes. The absence of eIF2α from the translation machinery is compensated by another initiation factor, eIF2A. Using human DRG explants and nerves obtained from organ donors, we first demonstrate that 24-hour treatment with MGO at 1uM, a concentration typically found in the serum of individuals with DNP, is sufficient to induce the integrated stress response as well as upregulate the expression of eIF2A. In vitro, dissociated human sensory neurons show elevated levels of p-eIF2α and eIF2A, particularly in putative nociceptors. We then used a newly characterized global knockout eIF2A mouse to assess the contribution of eIF2A to MGO-induced pain hypersensitivity. Intraplantar injections of MGO (2, 7.2, and 20 ng) produced mechanical pain hypersensitivity in wild-type animals but not in eIF2A-null mice. Similarly, intraperitoneal administrations of MGO (5 mg/kg) produced tactile allodynia in wild-type mice but failed to do so in eIF2A-null animals. To further confirm the role of eIF2A in ISR-mediated pain, a single hindpaw injection of tunicamycin (10 ug), a potent ISR inducer, established chronic mechanical hypersensitivity in wild-type animals while the eIF2A knock-out animals did not show a change in mechanical sensitivity. Further biochemical analysis confirmed an increase in p-eIF2α and eIF2A in the sciatic nerve and DRG of MGO-treated animals. Our findings show that MGO induces the ISR in human sensory neurons, DRG explants, and nerves at a concentration associated with neuropathic pain in diabetic patients. Our work indicates that eIF2A likely regulates the translation of proteins associated with mechanical pain hypersensitivity following MGO and that these proteins may represent new targets for DNP.

Disclosures: M. Yousuf: None. **J.M. Mwirigi:** None. **C.D. Garcia:** None. **M.R. Parks:** None. **S. Shiers:** None. **A.A. Komar:** None. **Z. Campbell:** None. **G. Dussor:** None. **T.J. Price:** None.

Poster

P399. Peripheral Mechanisms of Neuropathic Pain Including Human Tissues

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P399.08

Topic: D.03. Somatosensation – Pain

Support: Medical Research Council grant MR/S003428/1
Versus Arthritis grant 21544
Versus Arthritis grant 21543
Pain Relief Foundation Liverpool
Eli Lilly & Co

Title: Igg from fibromyalgia patients causes hyper-excitability of peripheral sensory neuronsin mice

Authors: *M. R. ISRAEL[1], N. VASTANI[1], S. SENSI[2], C. GENTRY[1], A. GOEBEL[3], S. BEVAN[1], D. A. ANDERSSON[1];
[1]King's Col. London, London, United Kingdom; [2]Univ. of Liverpool, Liverpool, United Kingdom; [3]Walton Ctr. NHS Fndn. Trust, Liverpool, United Kingdom

Abstract: Background: Fibromyalgia is a syndrome characterised by widespread pain, fatigue and mental fog in which 40 - 60% percent of patients also experience parathesiae and pain exacerbated by changes in temperature. We have recently shown that some of the symptoms, including altered responses to nociceptive pressure, cold and non-stimulus evoked symptoms such as fatigue can be faithfully recapitulated in mice (Goebel et al, 2021). Mice administered with patient derived IgG have decreased thigh pressure and cold evoked thresholds compared to healthy control treated mice. The mechanism that underlies these pain behaviours is unknown however patient derived IgG is accumulated in dorsal root ganglion (DRG) tissue in these passive transfer experiments. Furthermore, both patients and mice treated with patient IgG showed a decrease in intra-epidermal nerve fiber density compared to healthy controls. We hypothesised that some of the patients symptoms are caused by changes in excitability and responsiveness to stimuli (mechanical, cold) of peripheral sensory neurons. **Methods:** IgG was isolated from FM patients (n = 4) and healthy control serum (n = 4). Female C57Bl/6 mice were treated with 8 mg (0.4 ml, i.p) patient -derived IgG or healthy control IgG and assessed for pressure and cold sensitivity. We then used whole cell patch clamp and *ex vivo* nerve recordings from cultured dorsal root ganglion cells and skin - saphenous preparations. **Results:** DRGs isolated and cultured from mice administered with IgG were broadly hyper-excitable compared with HC treated DRG. Specifically, FM DRG had a decreased rheobase, increased input resistance and fired more action potentials per ramp stimulus. *Ex vivo* sensory nerve fibers from FM treated animals were also broadly more excitable than HC treated. Canonical nociceptors, Aδ; and C-mechano fibers have decreased mechanical thresholds but do not fire significantly more action potentials per stimulus. There is a significant increase in the proportion of C fibers that respond to cold stimulus. Low threshold mechano receptors (Aß-SA, Aß-RA) have significantly increased responses to mechanical stimulation. A subset of Aß fibers developed *de*

novo cold sensitivity in FM treated animals. **Conclusions:** In this study, we define the precise changes in peripheral nerve excitability that are caused by auto-reactive IgG in FM patients. Mice administered with patient derived IgG have hyperexcitable sensory neurons. Most significantly, we have identified a subset of Aß fibers that have developed cold sensitivity after IgG treatment. Comprehensive changes in excitability may explain the diverse symptomology of patients.

Disclosures: M.R. Israel: None. **N. Vastani:** None. **S. Sensi:** None. **C. Gentry:** None. **A. Goebel:** None. **S. Bevan:** None. **D.A. Andersson:** 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.; Eli Lilly & Co.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.01

Topic: D.05. Olfaction and Taste

Support: NIA AG-049937A
NIH-NIDCD R01 5R01DC014487-05

Title: Basal forebrain GABAergic projections to the olfactory bulb are rapidly recruited by odor encounters in a stimulus specific manner

Authors: *P. S. VILLAR[1], R. C. ARANEDA[1], D. ALBEANU[2];
[1]Univ. of Maryland, College Park, MD; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The output of the olfactory bulb is tightly regulated by top-down signals. Among these, a heterogeneous collection of neurons in the basal forebrain broadly integrates cortical and subcortical afference, and broadcasts neuromodulatory and GABAergic input throughout all bulb layers. To date, however the dynamics and functional roles of these projections in controlling olfactory representations remain unknown. Here, we used multiphoton calcium imaging in awake mice to investigate the recruitment of these descending GABAergic projections in olfactory processing. GABAergic boutons were spontaneously active across bulb layers, exhibiting brief activity bouts in naïve mice. Boutons of the same axonal branch showed significantly higher correlation than those belonging to different axons, confirming their homogeneous activation. Odor presentation resulted in sparse responses across different bulb layers, in an odorant and concentration specific manner. Across multiple odorants, boutons responded by either enhancement or suppression of baseline activity, while mixed-type responses were rarely observed. The dynamics of these calcium signals were similar to the odor responses

exhibited by bulbar glutamatergic projections originating in the piriform cortex, characterized by fast onset and diverse decay times. Since both glutamatergic and GABAergic feedback projections share the same targets in the bulb, our results suggest that the bulb output is under the influence of descending signals able to rapidly change their polarity. In ongoing experiments, we are assessing the contribution of the olfactory cortex in carrying predictive signals and shaping the odor representations of these GABAergic inputs in task-engaged animals, in familiar odor scenes and to novel, unexpected stimuli. Our results indicate that the basal forebrain GABAergic projections to the bulb are rapidly recruited by odor encounters and develop diverse and stimulus specific responses.

Disclosures: P.S. Villar: None. **R.C. Araneda:** None. **D. Albeanu:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.02

Topic: D.05. Olfaction and Taste

Support: DARPA HR0011-18-2-0024
CRCNS: NSF 1724221

Title: The effect of neuromodulation of the olfactory bulb on cortical odor processing.

Authors: *K. MAMA[1], O. D. ESCANILLA[4], M. EINHORN[2], M. LITVACK[1], A. MEZHERITSKIY[1], C. LINSTER[3];
[2]Psychology, [3]CPL & Neurobio. and Behavior, [1]Cornell Univ., Ithaca, NY; [4]Binghamton Univ., Binghamton, NY

Abstract: Sensory and cortical neural networks are strongly interconnected and reciprocally affect each other when processing information. In the olfactory system, modulation of bulbar neural dynamics by various neuromodulators affects learning and memory as measured behaviorally. Because odor memories are likely to be formed and stored in networks postsynaptic to bulbar networks, we ask how bulbar neural dynamics affect cortical odor representation. We show how cortical odor representations can be predictive of behavioral measures pertaining to memory stability, duration, and specificity, as predicted by our computational modeling studies. Behaviorally and computationally, dynamics in bulbar networks have been shown to strongly modulate cortical readout and plasticity.
In our study, rats are passively exposed to a set of five odorants, each presented five times in a randomized order over a thirty-minute period, both before and after neuromodulation of the olfactory bulb. In each rat, we record field potentials and unit responses to odors in the olfactory bulb and cortex simultaneously using surgically implanted recording electrodes. To modulate

bulbar dynamics, we locally manipulate cholinergic activity by infusing the olfactory bulb either with saline, cholinergic antagonists, or cholinergic agonists. To eliminate motivational confounds (for this first study) and to have access to respiration signals, rats were tested in a custom-designed cage equipped with pressure sensors and olfactometer inputs. This enables the recording of respiratory activity and allows for precise odor delivery without the need for prior behavioral conditioning. With this setup, we observed changes in cortical odor responses and dynamics driven by changes in bulbar dynamics. We are analyzing to what extent these are accompanied by plasticity-driven effects in response to repeated odor stimulation. So far, predictions from behavioral studies and computational modeling are confirmed. The next step will be to use food motivated odor learning paired with recordings and manipulations of bulbar dynamics.

Disclosures: K. Mama: None. **O.D. Escanilla:** None. **M. Einhorn:** None. **M. Litvack:** None. **A. Mezheritskiy:** None. **C. Linster:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.03

Topic: D.05. Olfaction and Taste

Support: NSF IOS #1755284
NIH (NIDCD) #R01DC017234
NIH (NINDS) #R24NS098536

Title: In vivo calcium imaging of accessory olfactory bulb mitral cells during social interactions

Authors: S. T. BYERS, *K. J. MONK, I. G. DAVISON;
Dept. of Biol., Boston Univ., Boston, MA

Abstract: The vomeronasal system plays a pivotal role in guiding social behavior through the sensing of behaviorally-relevant chemosignals (e.g., pheromones). While responses to social cues have been characterized in the accessory olfactory bulb (AOB), much less is known how such chemosignals are represented during unrestrained interactions that elicit natural social behaviors. Here, using miniscope Ca2+ imaging of AOB activity in freely moving mice, we assess how the AOB encodes sensory information during social investigations of male and female conspecifics from various background strains. First, in constrained interactions, experimental animals are allowed to investigate restricted body regions of various partners, allowing us to image sensory-evoked responses to facial or anogenital regions associated with distinct chemosignals with high spatiotemporal precision. Second, we allow mice to freely investigate and interact with a range of probe animals while recording evoked activity. In both

cases, while a subset of glomeruli show strongly selective activation for male and female probe animals, most show mixed responsiveness. We also find selective activation to chemosignals located on different body regions, reflecting the distinct cues secreted at these locations. We analyzed the microstructure of social interactions and found that mice tend to structure their investigations as sweeps from nose to tail over one to two seconds, suggesting there may be preferred sequences of social investigations that could serve to bias incoming sensory information. However, the rapidity of investigation sequences contrasts with the prolonged timescale of AOB neural activation, suggesting that the AOB and vomeronasal system integrate information across extended timescales rather than directly mapping individual sensory events onto discrete behavioral responses. Together, these findings extend our understanding of how the AOB represents behaviorally-relevant chemosignals in support of social behavior.

Disclosures: S.T. Byers: None. **K.J. Monk:** None. **I.G. Davison:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.04

Topic: D.05. Olfaction and Taste

Support: NIH Grant 1U01NS090498-01
NIH Grant U19NS107464

Title: Combining natural and synthetic stimulation to unravel the rules governing sensory processing

Authors: *J. V. GILL[1], D. ASUA[2], S. SHOHAM[2], D. RINBERG[1];
[1]Neurosci. Inst., [2]Tech4Health Inst., New York Univ. Langone Hlth., New York, NY

Abstract: Animals rely on their senses for survival. The speed and precision of sensory guided behavior is enabled by multiple stages of computation from the peripheral to the central nervous system. However, what computations are performed, and how the functional connectivity of sensory circuits support perception is still largely unknown. The mouse olfactory bulb provides an ideal model for investigating the connection between the structure of connectivity and functional output, given the ease of access to both the input (glomeruli) and output (mitral cell) layers for recording and manipulation. To this end we developed a system for simultaneous large-scale 2-photon calcium imaging and holographic stimulation in the olfactory bulb of awake mice, permitting interrogation of circuit function with single neuron and action potential resolution. Using this technique, we recently demonstrated that manipulating activity in small groups of mitral cells, the projection neurons of the mouse olfactory bulb, can have a large impact on perception. Yet, it is unknown what computation is performed by the bulbar network,

and how odor representations are reshaped by the interactions between excitatory projection neurons and inhibitory interneurons.
To address this knowledge gap, we first explored the structure of encoding at the level of mitral cells in awake mice, presenting a large battery of odors while recording the responses of populations of mitral and granule (inhibitory) cells using 2-photon calcium imaging. From these responses we found a low-dimensional projection which well-preserved the distances in odor tuning between neurons. We then probed the structure of functional connectivity between mitral cells by individually activating neurons using holographic photostimulation while measuring the effect on the local population. While mitral cells are predominantly interconnected by inhibitory interneurons, without anatomical evidence for direct excitatory connections, we surprisingly observed a mixture of excitatory and inhibitory influence exerted between mitral cells. In ongoing experiments and analyses we attempt to relate the sign and strength of coupling between mitral cells to distances in odor tuning to determine how local connectivity reshapes odor representations.

Disclosures: J.V. Gill: None. **D. Asua:** None. **S. Shoham:** None. **D. Rinberg:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.05

Topic: D.05. Olfaction and Taste

Support: 1R01DC018516

Title: Neurogenesis of Calretinin-expressing neurons in the mouse olfactory bulb

Authors: *T. R. BRECHBILL[1], T. KUNKHYEN[2], C. E. CHEETHAM[3];
[2]Neurobio., [3]Dept. of Neurobio., [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Throughout adulthood, neuronal progenitors are born in the mouse subventricular zone (SVZ) and migrate to the olfactory bulb (OB) via the rostral migratory stream. There, the immature neurons migrate radially and differentiate into several molecularly and functionally distinct neuronal types. One of these is the calretinin (CR)-expressing neurons, a subtype of GABAergic interneurons. CR+ neurons have very high densities within the glomerular layer (GL) and the granule cell layer (GCL) and much is unknown about their role in olfactory function. The goal of this study was to determine whether OB CR+ neurons are dependent on sensory input from OSNs for their survival, and whether additional CR+ neurons are generated when sensory input is restored.
To test this, we specifically ablated olfactory sensory neurons in the OE, which provide sensory input to the OB and themselves regenerate, using methimazole (MMZ). 75 mg/kg of either MMZ

or sterile saline was injected intra-peritoneally in 8-week-old C57BL/6J mice. Mice were transcardially perfused at 7, 14 or 35 days post-injection to assess the effect of loss followed by regeneration of sensory input. OBs from 3-5 mice per time point were dissected and cut into 40-micron coronal sections. We used sections 25% (anterior), 50% (central) and 75% (posterior) along the anterior-posterior axis. Immunohistochemical staining for calretinin was performed and sections were imaged using a large area scanning widefield fluorescence microscope (Nikon-90i) using a 10x objective. We determined the density of CR+ neurons in the GL, EPL and GCL, using DAPI fluorescence to define each layer.
Interestingly, 7 days post-injection, CR+ neuron density was higher in MMZ-treated than in saline-treated mice in both the GL and EPL ($P < 0.001$ for effect of MMZ treatment, 2-way ANOVA). However, CR+ neuron density was similar in the GL and EPL of MMZ- vs. saline-treated mice at 14- and 35-days post-injection. In contrast, there was no significant difference in CR+ neuron density in the GCL between MMZ- and saline-injected mice. Between 7 and 14 days post-MMZ, CR+ neuron density decreased significantly in the GL ($P = 0.027$) and showed a strong tendency to decrease in the EPL ($P = 0.069$). We are currently determining the contribution of changes in CR expression, integration of newborn CR+ neurons and cell death on changes in CR+ neuron density in the GL and EPL.
Overall, our data suggest that GL CR+ neurons are not as reliant on OSN input for their survival as other GL GABAergic interneuron subtypes, such as dopaminergic neurons. Our data also highlight differences in the activity dependence of CR+ neuron density in the GL/EPL vs. the GCL.

Disclosures: T.R. Brechbill: None. **T. Kunkhyen:** None. **C.E. Cheetham:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.06

Topic: D.05. Olfaction and Taste

Support: K99 DC017754
R01 DC016289
R01 DC014453

Title: Nonlinear representations of odor mixtures in feedforward and feedback pathways of the olfactory system

Authors: *J. D. ZAK, V. N. MURTHY;
Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Sensory systems are organized hierarchically. Early stages efficiently format transduced signals and successive processing steps perform more complex computations to extract relevant sensory representations. This feedforward hierarchy is broken early in the olfactory system, where dense feedback projections from cortical regions arrive at the olfactory bulb. In fact, the descending inputs from the cortex to the olfactory bulb outnumber the afferent inputs it receives from the sensory periphery. Therefore, revealing how these projections, both feedforward and feedback, contribute to the coding of complex stimuli is necessary for understanding the sensory processing that takes place in both the olfactory bulb and cortex. We expressed calcium indicators in olfactory sensory neurons, or the piriform cortex, and used multiphoton imaging to measure the stimulus-response properties of feedforward and feedback projections to the olfactory bulb in awake and freely breathing mice. We used two odor delivery paradigms that revealed surprising aspects of how odors and their mixtures are represented in cortical projections. First, monomolecular odors spanning a concentration range of more than three odors of magnitude evoked responses in feedback axons that, as a population, and like feedforward inputs, increased with concentration. However, at the level of individual boutons, we observed odor responses that had complex, non-monotonic concentration dependence that often reflected a preferred range. We next imaged bouton responses to odor mixtures that contained between 2 and 14 components. Feedback bouton responses were highly sublinear and even more so than feedforward inputs to the olfactory bulb. The activity in feedback projections typically mirrored the response of a single mixture component. Finally, we compared how similar mixtures, based on their component identities and activity patterns, are represented in feedforward and feedback projections. These results suggest that the piriform cortex may be providing highly demixed sensory information to the olfactory bulb, dominated by individual odor components. Future studies will focus on how the nonlinear activity patterns in cortical projections are inherited by olfactory bulb projection neurons.

Disclosures: J.D. Zak: None. **V.N. Murthy:** None.

Poster

P419. Olfactory Coding: Olfactory Bulb I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P419.07

Topic: D.05. Olfaction and Taste

Support: NIH-NIDCD R01 5R01DC014487-05
NSF IOS-1656830

Title: A non-canonical feedforward pathway for computing odor identity

Authors: *H. CHAE[1], A. BANERJEE[1,2], M. DUSSAUZE[1,2], D. F. ALBEANU[1,2];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Cold Spring Harbor Lab. Sch. for Biol. Sci., Cold Spring Harbor, NY

Abstract: Elucidating the neural circuits that enable robust odor identification remains a fundamental challenge in olfaction. The prevailing view is that odor identity is computed within the piriform cortex (PC), drawing from mitral cell input from the olfactory bulb (OB). Here, we assessed the validity of this model by measuring the interplay between feedforward and feedback signals across different olfactory areas in representing odor identity by leveraging cell type specific analysis. In awake mice, we monitored the odor responses of the two classes of OB outputs, the mitral and tufted cells via multiphoton microscopy, as well as from their major cortical targets, the piriform cortex and the anterior olfactory nucleus (AON) respectively using tetrode micro-drives. We find that tufted cells, which strongly innervate the AON, substantially outperform mitral cells in decoding both odor identity and intensity, as well as in intensity invariant decoding, acting in a largely feedforward fashion. Cortical-bulbar feedback from the piriform cortex regulates the activity of mitral cells while the AON preferentially regulates the activity of tufted cells, matching biases in feedforward connectivity. Moreover, piriform cortex feedback specifically restructures the mitral cell responses leaving tufted cell responses largely unaltered, while feedback from the AON preferentially controls the gain of tufted cell odor representations. Finally, preliminary electrophysiology recordings indicate that the AON neural ensembles outperform piriform cortex ensembles in decoding both odor identity and concentration information, thus reflecting differences between their tufted and mitral cell inputs. These results indicate that, unexpectedly, the non-canonical tufted cell-to-anterior olfactory nucleus pathway plays a major role in mediating odor recognition and discrimination and re-define our understanding of how and where odor identity and intensity are computed across the brain

Disclosures: H. Chae: None. **A. Banerjee:** None. **M. Dussauze:** None. **D.F. Albeanu:** None.

Poster

P577. Sensory Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P577.01

Topic: F.01. Neuroethology

Support: TPCN Grant 1R90DA043849-01

Title: Transformation of Whisker Touch Representations from Layer 4 to Layer 2 in Primary Vibrissal Somatosensory Cortex

Authors: B. VOELCKER, R. PANCHOLI, L. RYAN, S. P. PERON;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Sensory input arrives from thalamus in cortical layer (L) 4. From L4, information flows predominantly to superficial layers, so that L4 to L2 constitutes one of the earliest cortical feedforward networks. Despite extensive study, the transformation performed by this network remains poorly understood. We use volumetric two-photon calcium imaging in L2-4 of primary vibrissal somatosensory cortex (vS1) to densely sample activity as transgenic mice expressing GCaMP6s in excitatory neurons perform an object localization task with two whiskers. We find that the touch response sparsens superficially and becomes more reliable. Touch-evoked pairwise correlations among the whole touch cell population increase superficially. In addition, L3 and L2 contain ensembles of ~20 extremely highly correlated neurons, that reliably respond to specific types of touch. Our results show that from L4 to L2, cortex transitions from a dense probabilistic code to a sparse and robust ensemble-based code.

Disclosures: B. Voelcker: None. **R. Pancholi:** None. **L. Ryan:** None. **S.P. Peron:** None.

Poster

P577. Sensory Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P577.02

Topic: F.01. Neuroethology

Support: NIH Grant R01 NS117536

Title: The role of individual barrels in object localization behaviors

Authors: *L. RYAN[1], M. LAUGHTON[1], A. SUN-YAN[1], R. PANCHOLI[1], B. VOELCKER[1], S. P. PERON[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: In the rodent, the vibrissae constitute a crucial sensory organ, with around twenty percent of the somatosensory cortex dedicated to processing information from the whiskers. This cortical area projects to higher order sensory and motor areas and is likely a crucial node for sensorimotor integration during behavior. However, the behavioral role of vibrissal primary somatosensory cortex (vS1, or 'barrel cortex') is unclear. Large-scale lesions, which targeted vS1 but typically included additional brain areas, such as secondary vibrissal somatosensory cortex, suggest that vS1 is necessary for rodent performance on whisker-based object localization tasks where mice must discriminate between contacts at two or more positions. In object detection tasks, cortical lesions produce a transient behavioral decrement, but mice recover within a day or two. The large extent of these lesions, as well as the fact that they require

surgical intervention which necessitates extensive recovery, makes these results difficult to interpret. Past optogenetic silencing and pharmacological inactivation experiments also lack barrel-scale resolution. Thus, the role of vS1, and of individual barrels, in localization behaviors remains unclear. In gap crossing tasks, barrel-scale lesions result in performance decrement. We lesion individual barrels in mice performing an object localization task with a single spared whisker. Mice expressing GCaMP6s and implanted with a cranial window perform an object localization task with a single spared whisker in which they touch multiple anterior and posterior object positions, reporting pole position by licking one of two lickports. We target and lesion the individual vS1 barrel of the spared whisker (typically, C2) using prolonged exposure to a high-powered femtosecond laser source. Lesions are confined to vS1, and lesion extent ranged from 0.07 +/- 0.02 mm^3 (mean +/- S.D.; n = 4, volume of a single barrel: 0.07 mm^3). Barrel lesions result in an irreversible decline in behavior in mice trained on an object localization task. Mice are retrained on a detection task following the lesion. A separate cohort of mice trained on a go/no go vibrissal detection task showed no behavioral decrement following vS1 lesions. Thus, individual vS1 barrels are critical for animals to perform discrimination, but not detection, tasks with the whisker whose stimulation drives the lesioned barrel. We demonstrate a reproducible and non-invasive lesioning technique that allows for high-throughput experiments testing areal necessity and the contribution of individual cortical areas to the distribution of behaviorally relevant information throughout cortex.

Disclosures: L. Ryan: None. **M. Laughton:** None. **A. Sun-Yan:** None. **S.P. Peron:** None.

Poster

P577. Sensory Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P577.03

Topic: F.01. Neuroethology

Support: NIH Grant R01 NS117536

Title: Cortical dynamics during optical microstimulation task training

Authors: *R. PANCHOLI[1], L. RYAN[1], B. VOELCKER[1], S. P. PERON[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: Theoretical studies of cortical plasticity have predicted that repeated activation of a group of neurons should drive a host of changes in primary sensory cortex. However, technical limitations with stimulation and recording techniques, as well as noise and plasticity introduced at processing stages prior to cortex, have made it difficult to interpret cortical network changes resulting from sensory stimulation. Here, we train transgenic mice expressing GCaMP6s in cortical pyramidal neurons to perform an optogenetic pulse count discrimination task. Mice must

use activity evoked in layer 2/3 of primary vibrissal somatosensory cortex (vS1) to report whether the number of optogenetic LED pulses presented to cortex was high or low. As mice learn to perform this task, we use two-photon calcium imaging to track vS1 neural dynamics across learning. We find distinct populations of neurons whose activity declines, remains stable, or increases over the course of training. These changes are consistent with theoretical models of repeated cortical stimulation showing increased feedback inhibition for highly responsive neurons. A decoding analysis further allows us to track choice-related activity dynamics across learning and reveals neurons whose activity is highly correlated with animal choice. Future experiments will explore the behavioral impact of neurons with varying levels of stimulus-evoked and choice-related activity.

Disclosures: R. Pancholi: None. **L. Ryan:** None. **B. Voelcker:** None. **S.P. Peron:** None.

Poster

P577. Sensory Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P577.04

Topic: H.03. Decision Making

Support: NIH/NNDS R01 Grant NS107599
Whitehall Foundation Grant 2017-05-71

Title: Laminar specificity of learned sensory, sensory-motor, and motor signals in frontal cortex during a whisker detection task

Authors: *B. ZAREIAN[1], A. LAM[2], E. ZAGHA[1];
[1]Dept. of Psychology, Univ. of California Riverside, Riverside, CA; [2]Biomed. Sci., Univ. of California, Riverside, Riverside, CA

Abstract: Laminar specificity of learned sensory, sensory-motor, and motor signals in frontal cortex during a whisker detection taskWhisker motor cortex (wMC) has been shown to be important in lateralized attenuation of sensory information and sensory-motor transformation in a selective whisker detection task in mice (Aruljothi et al. 2020, Zareian et al. 2021). Laminar profile of attenuation and how the sensory signal leads to generation of a motor signal remains elusive. To explore the answer, we used laminar electrophysiological recording during behavior to characterize laminar propagation of sensory signals within the frontal cortex and interhemispheric propagation between bilateral frontal cortices. Importantly, we restricted our analyses to 100 ms post-stimulus where there is less interference from movement. We show that the sensory signal is similar within supragranular layers between the target and distractor wMC, yet different in infragranular layers (Target wMC>Distractor wMC). Also, within the target hemisphere, we found proportionally more motor than sensory signals in layer 6. Furthermore,

we found that choice signals appear earlier in infragranular layers. Regarding interhemispheric communication, more target signal reaches the distractor wMC overall than distractor signal reaching target wMC. However, proportionally there is more percent attenuation of sensory signal from target wMC to distractor wMC than the opposite direction. These findings suggest that within wMC, sensory signals in supragranular layers are largely context-independent, with task-dependent sensory and choice signals prominent in layer 5 and large motor signals in layer 6. Ongoing studies are comparing cortical propagation to subcortical pathways.

Disclosures: B. Zareian: None. **A. Lam:** None. **E. Zagha:** None.

Poster

P577. Sensory Systems I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P577.05

Topic: F.01. Neuroethology

Support: SCNIP Studentship Award
Alberta Graduate Excellence Scholarships
CIHR Project Grant PJT-173511
Wings for Life

Title: Population-level fiber photometry recording of A11 activity in different behavioral contexts

Authors: *E. M. S. THURSTON[1,7,2], L. A. MOLINA[2], V. A. LE[3], N. CHENG[4,5], K. MURARI[3,7], P. J. WHELAN[6,7,5];
[1]Dept. of Neurosci., [2]Cumming Sch. of Med., [3]Dept. of Electrical and Software Engin., [4]Dept. Comparative Biol. and Exptl. Med., [5]Fac. of Vet. Med., [6]Univ. of Calgary, Calgary, AB, Canada; [7]Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: The dopaminergic A11 nucleus is found in many vertebrate species. Previous work from our lab has shown that photostimulation of the dopaminergic A11 population in mice increased locomotor behaviors. In contrast, avian research has shown an increase in *fos* expression following courtship singing or copulation behaviors. We investigated activity levels of the A11 region during sociosexual, locomotor, or arousal-based behaviors. C57BL6/J male mice 8-10 weeks old were injected with GCaMP6s (pAAV.CAG.GCamP6s.WPRE.SV40) into the A11 region and implanted with an optrode to record fluorescence as a proxy for neuronal activation. Following a 4-week incubation period, mice were handled and habituated to the testing conditions, and then tested in a variety of behavioral paradigms while synchronized photometry recording was conducted: open field, elevated plus maze, and presented with novel stimuli in a custom testing arena including a male or female C57 mouse or a novel object. Mouse

testing order was randomized to minimize order effect. Behavioral data were extracted from videos of these tests (TopScan or a custom tracking software), and behaviors were correlated to A11 calcium transients using a custom MATLAB script. Our results suggest that the A11 region is associated with lower-anxiety behaviors such as grooming, or exploration in an elevated plus maze test. In addition, A11 calcium transients were increased following the presentation of a novel object, but calcium transients were increased or decreased depending on social context. This research is the first to investigate population-level activity of this region correlated with a variety of behaviors. Our study highlights the context-specific activity patterns of the A11 across a standard behavioral battery of tests.

Disclosures: E.M.S. Thurston: None. **L.A. Molina:** None. **V.A. Le:** None. **N. Cheng:** None. **K. Murari:** None. **P.J. Whelan:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.01

Topic: F.04. Stress and the Brain

Support: E.E. Just Fellowship
Simons Society of Fellows

Title: Exploring the inheritance of Threat Sensitivity

Authors: *A. KIM[1], B. J. MARLIN[2];
[1]Columbia Univ., New York, NY; [2]Neurobio., Columbia Univ. Zuckerman Inst., New York, NY

Abstract: Alexis KimSFN AbstractJuly 15th, 2021Abstract Transgenerational epigenetic inheritance (TEI) is the ability of an organism to transmitbiological markers from one generation (F0 generation) to the subsequent generation (F1generation) without perturbing the intrinsic genome. The inheritance of these markers may alterthe phenotype of succeeding generations and offers a channel for the transmission ofenvironmental information. TEI has been exhibited in the inheritance of paternal stress wherebymale mice conditioned to a specific odor with a painful shock, demonstrated heightenedsensitivity and aversive behavior toward the odor. Consequently, we have found that theoffspring of the olfactory fear conditioned (OFC) male mice, exhibited increased olfactorysensory neurons (OSN) attuned to the odor that their fathers have feared, in comparison to thecontrol. To further explore the gravity of this inheritance, we examine the arousal of the F1generation upon presentation of various odors, including the odor that their forefathers havelearned to fear. Arousal marks a change in internal state preluding perception and is keenlymediated by noradrenaline, released by the Locus Coeruleus (LC). Arousal has been linked tostress, transmitted by inputs from the central nucleus of the amygdala (CeA)

projecting to theLC. We intend to measure the state of arousal using pupillometry on OFC males and their F1offspring. Pupil dilation is directly mediated by noradrenergic projections by the sympatheticnervous system. By verifying arousal in the presence of the odor, the stress response may berecruited supporting the inheritance of stress.**Methodology**To establish the F0 population, we plan on conditioning 2 male (C57BL/6J) mice, test subjectand a control, to an odor with a total of 5 odors for the study (n = 10). Four of the five odors arespecific to one receptor in the main olfactory epithelium, while the other odor is well known tobe innately aversive. The F0 generation will then breed with naive wildtype females to generatethe F1 generation. Both F0 and F1 generations will then undergo a behavior paradigm wherethey are presented various odors, including the conditioning odor, at random and their pupilsize will be measured throughout the experiment.**Significance**This study aims to understand the ways in which a novel aversive behavior in one generationmay become innate in the next generation.

Disclosures: A. Kim: None. **B.J. Marlin:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.02

Topic: F.04. Stress and the Brain

Support: NIMH RO1MH096093
Harvey Family Endowment

Title: Brain-wide imaging of network dynamics after chemogenetic activation of the locus coeruleus by MEMRI: Implications of tonic noradrenergic activity on brain-behavior.

Authors: *T. W. USELMAN[1], R. E. JACOBS[2], E. L. BEARER[1,3];
[1]Dept. of Pathology, Univ. of New Mexico - Hlth. Sci. Ctr., Albuquerque, NM; [2]Zilkha Neurogenetic Inst., USC Keck Sch. of Med., Los Angeles, CA; [3]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Monoaminergic systems including the noradrenergic system (NS) regulate brain-wide physiology in responses to stress, reward, and many other experiences. Dysregulation of normal NS activity is associated with altered states i.e. anxiety/depression, or cocaine use. Activity of noradrenergic neurons in the locus coeruleus (LC) releases norepinephrine in distal brain regions ultimately modulating post-synaptic pyramidal and interneuron firing in salience information processing. Our hypothesis is that tonic activation of LC will increase activity in stress- and reward-mediating regions, e.g. BNST, Hyp, Amg, VTA, and NAc. Here, we investigate chemogenetic activation (hM3Dq) of LC by performing behavioral recordings in parallel with brain-wide manganese-enhanced MRI (MEMRI). Intraperitoneal injections of paramagnetic

Mn(II) (0.3 mmol/kg) highlights brain activity in awake behaving mice by T1-weighted MRI. Adult mice were subjected to either longitudinal MEMRI (n = 12) or open field behavior (n = 8) to assess the effects of CNO±hM3Dq expression. Naïve C57BL6J mice were first video recorded and behavioral parameters tracked, before and after intraperitoneal administration of CNO (3 mg/kg), using Noldus Ethovision XT15. All mice underwent stereotactic injection of CAV2-PRS-hM3D(Gq)-mCherry targeting the LC (AP = -5.4, ML = ±0.80, DV = -3.80). Behavior was again recorded before and after CNO 4-8 weeks post-injection. Another hM3D expressing group was imaged for Mn(II) accumulation before and after CNO (3 mg/kg). A third control group was imaged using the same MEMRI procedures to examine the effect of CNO without hM3D expression. The effect of CNO was monitored by the pupillary light response. Expression of the transgenes was confirmed by microscopy of brain sections through LC. Preliminary data show that CNO alone influenced the pupillary light response, which increased with hM3Dq expression. Expression was localized to bilateral LC. Mn(II) accumulation increased signal intensity in the hippocampus. CNO alone reduced exploratory behavior. MEMRI images are being analyzed for neural activity patterns by statistical parametric mapping (SPM), segmentation of 90 brain regions, cross-correlation analysis, and Louvain community detection. Principal component analysis of behavioral parameters will be compared statistically to patterns of regional brain activity to correlate the two modalities. We conclude that this imaging/behavior strategy will directly test our hypothesis and provide insight into the effect of NS on brain-wide neural activity including critical regions of the salience network. Supported by NIMH RO1MH096093.

Disclosures: T.W. Uselman: None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.03

Topic: F.04. Stress and the Brain

Support: DP2-NS115764

Title: A novel intersectional tool to study locus coeruleus subpopulations

Authors: *A. C. HUGHES, G. POLLARD, L. A. SCHWARZ;
St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Advances in genetic profiling have furthered our understanding of the neuronal subpopulations present in the brain. As these classifications grow in specificity, so too does our need to target these unique populations selectively. Current intersectional strategies target these subpopulations by utilizing viral constructs to deliver a payload that is expressed after

subsequent recombination events mediated by different recombinases (i.e., Cre and Flp). However, these targeting approaches often require multiple viral constructs, bulky silencing regions, or complex cloning strategies, thus limiting their use and adaptability. I have developed a novel, single-construct intersectional targeting strategy that is easily customizable and allows for the delivery of large payloads. I will use this ConVERGD (Conditional Viral Expression by Ribozyme Guided Degradation) strategy to target specific neurons within the locus coeruleus (LC). As the primary source of norepinephrine (NE) for the adult brain, the LC is a major regulator of diverse arousal states. Less known is how the genetic diversity of the LC neurons contributes to this regulation. Using ConVERGD constructs, I am able to target a novel subpopulation of LC neurons producing both NE and the stress-related endogenous opioid dynorphin (Dyn). These studies showcase ConVERGD as a versatile tool capable of targeting genetic subpopulations of neurons. Through this work, we hope to reveal Dyn+/NE+ LC neurons as a clinically relevant neuronal population that contributes to the aversive attributes of stress.

Disclosures: A.C. Hughes: None. **G. Pollard:** None. **L.A. Schwarz:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.04

Topic: F.04. Stress and the Brain

Support: 1R56MH121918-01A1

Title: Sex-specific effects of stressor exposure on coping behavior and locus coeruleus activity in the defensive shock probe burying task

Authors: *J. TKACZYNSKI, B. DUFFY, D. F. MANVICH, D. J. CHANDLER;
Dept. of Cell Biol. & Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: Stress is a physiological state that occurs in response to threatening stimuli that is characterized by specific endocrine and behavioral changes that aid in survival. One structure in the brain that is stress-responsive and contributes directly to anxiety-like behavior and affect is the noradrenergic nucleus locus coeruleus. Previous observations from our laboratory have shown that an acute stressor, a single episode of combined physical restraint and predator odor exposure, leads to long-lasting changes in LC physiology and anxiety-like behavior in the open field test in adolescent rats. Interestingly, however, male and female rats show opposing changes in both measures. We propose that these differences in stress-induced anxiety-like behavior represent a sex-based difference in coping strategies. In order to investigate this, we plan to use the defensive shock probe burying task along with secondary physiological measurements of serum corticosterone concentration and locus coeruleus c-Fos expression. Preliminary data

suggest that males with a history of prior stressor exposure exhibit more active burying behavior, while females exhibit more passive avoidance behavior. Despite the sex-specific responses to stressor exposure in burying behavior, there are no apparent differences in serum corticosterone concentrations according to sex or treatment. We also hypothesize that males with a history of stressor exposure will show reduced c-Fos expression following the shock probe task, relative to control males, while females will show the opposite trend. These findings will help to clarify the role between sex, stress, and coping behavior.

Disclosures: J. Tkaczynski: None. **B. Duffy:** None. **D.F. Manvich:** None. **D.J. Chandler:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.05

Topic: F.04. Stress and the Brain

Support: NIH R01-170553
NIH R01-097498
Endowment for the Basic Sciences Innovation Initiative of the University of Michigan Medical School

Title: Stress related network activity in the intact adrenal medulla

Authors: *J. R. LOPEZ RUIZ[1], S. A. ERNST[2], R. W. HOLZ[3], E. L. STUENKEL[1,4];
[1]Mol. and Integrative Physiol., [2]Cell and Developmental Biol., [3]Pharmacol., Univ. of Michigan, Ann Arbor, MI; [4]Col. of Hlth. and Life Sci., Hamad Bin Khalifa Univ., Doha, Qatar

Abstract: The adrenal medulla plays a critical role in mammalian homeostasis and the stress response. It is populated by clustered chromaffin cells that secrete epinephrine or norepinephrine along with peptides into the bloodstream affecting distant target organs. Despite been heavily studied, the central control of adrenal medulla and in-situ spatiotemporal responsiveness remains poorly understood. For this work, we continuously monitored the electrical activity of individual adrenomedullary chromaffin cells in the living anesthetized rat using multielectrode arrays. We measured the chromaffin cell activity under basal and physiological stress conditions and characterized the functional micro-architecture of the adrenal medulla. Under basal conditions, chromaffin cells fired action potentials with frequencies between ~0.2 and 4 Hz. In the anesthetized animal, chromaffin cells were exclusively driven by sympathetic inputs coming through the splanchnic nerve. Furthermore, chromaffin cells were organized into independent local and distant networks in which cells fired in a specific order, with latencies from hundreds of microseconds to a few milliseconds. Electrical stimulation of the splanchnic nerve evoked the

exact spatiotemporal firing patterns that occurred spontaneously. Hypoglycemic stress, induced by insulin administration resulted in increased activity of a subset of the chromaffin cells. In contrast, respiratory arrest induced by lethal anesthesia resulted in an increase in the activity of the entire adrenal medulla before cessation of all activity. These results suggest a stressor-specific activation of adrenomedullary chromaffin cell networks and revealed a surprisingly complex electrical organization and circuitry that likely reflects the dynamic nature of the adrenal medulla's neuroendocrine output during basal conditions and during different types of physiological stress.

Disclosures: J.R. Lopez Ruiz: None. **S.A. Ernst:** None. **R.W. Holz:** None. **E.L. Stuenkel:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.06

Topic: F.04. Stress and the Brain

Support: NIH R37HD091280
DGE 1746891

Title: Defining neuronal subtypes and their connectivity in the nucleus incertus

Authors: *E. SPIKOL[1], J. CHENG[2], M. MACURAK[3], A. SUBEDI[3], M. E. HALPERN[2]; [1]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Mol. and Systems Biol., Dartmouth Geisel Sch. of Med., Hanover, NH; [3]Embryology, Carnegie Inst., Baltimore, MD

Abstract: The nucleus incertus (NI), an understudied brain region at the midline of the floor of the 4th ventricle, has been implicated in the response to aversive stimuli, and is the main site for producing the neurohormone Relaxin-3 (Rln3). Not all neurons in the NI produce Relaxin-3, but little is known about the differences between *rln3*-positive and *rln3*-negative populations. Larval zebrafish serve as a powerful vertebrate model to investigate these conserved neural pathways, and their transparency and genetic tractability are advantageous for monitoring and manipulating specific neuronal subpopulations. We found that a group of neurons that express *goosecoid 2* (*gsc2*) is located just anterior to *rln3* neurons in the zebrafish hindbrain. We used CRISPR/Cas9 targeted integration to generate transgenic lines for selective labeling and manipulation of *gsc2* and *rln3* expressing neurons to understand their identity, connectivity, and function. Anatomical, hodological and neurochemical characteristics of the *gsc2* and *rln3* neurons are consistent with NI identity. However, these adjacent neuronal subsets differ in their spontaneous activity and their responses to aversive stimuli as measured by genetically encoded calcium indicators. Additionally, *gsc2* and *rln3* neurons differ in their efferent and afferent connectivity: their axons

terminate within different regions along the dorsoventral extent of the midbrain interpeduncular nucleus (IPN). The NI is also thought to receive projections from the IPN in a reciprocal manner and is a proposed target of the dorsal habenula (dHb)-IPN neural pathway. Our data indicate that optogenetic activation of the dHb-IPN axis increases activity in *gsc2* neurons, but not in *rln3* neurons. Perturbation of these two distinct neuronal subgroups, by ablation or disrupting their activity, will provide additional information on their specialized functions and the role of the NI in the stress response.

Disclosures: E. Spikol: None. **J. Cheng:** None. **M. Macurak:** None. **A. Subedi:** None. **M.E. Halpern:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.07

Topic: F.04. Stress and the Brain

Support: NIH Grant 1R01MH122844

Title: 2-arachidonoylglycerol signaling in the lateral habenula: impacts on stress coping behavior and downstream immediate early gene expression

Authors: *H. R. WRIGHT[1], D. E. GINDER[2], J. L. RICHIE[1], Z. D. G. FISHER[1], A. M. BROWN[1], R. URRUTIA-CARMAGO[1], R. A. FUCHS[1], R. J. MCLAUGHLIN[1,2]; [1]Integrative Physiol. and Neurosci., [2]Psychology, Washington State Univ., Pullman, WA

Abstract: Individual differences in stress coping strategies impact the development of stress-related disorders and are orchestrated in part by activity of dopamine (DA) neurons in the ventral tegmental area (VTA) and serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN). The lateral habenula (LHb) regulates both DA and 5-HT activity via inhibitory neurons within the rostromedial tegmental nucleus (RMTg) that project to the VTA and DRN. Our previous data indicate that the endocannabinoid (eCB) 2-arachidonoylglycerol (2-AG) is recruited in the LHb during stress, and intra-LHb cannabinoid type-1 receptor agonist or inverse agonist administration increases avoidant and approach behavior respectively, suggesting that manipulating the concentration of 2-AG by interfering with its synthesis or metabolism may affect LHb-mediated regulation of behavioral responses. However, the role of LHb 2-AG in the expression of stress coping strategies and the downstream mechanisms underlying these effects remain unknown. Thus, we set out to determine if altering LHb 2-AG concentration during acute or chronic social defeat stress (SDS) affects coping behavior and downstream expression of the immediate early gene zif/268. Male Sprague Dawley rats (n=12/group) were implanted with bilateral cannula aimed at the LHb. Rats were randomly assigned to receive a microinfusion of

the 2-AG hydrolysis inhibitor MJN110 (0, 0.5, or 1 μg/side) prior to acute SDS exposure. In the chronic SDS experiment, rats underwent 7 daily SDS sessions and were randomly assigned to receive a microinfusion of the 2-AG synthesis inhibitor DO34 (0, 0.07, or 0.7 μg/side) prior to the last session. Sessions were video recorded for blinded analysis with deep learning pose-estimation and predictive classifier software to identify behaviors. Rats from the chronic SDS experiment were then euthanized 2 hr after the SDS session and brain tissue encompassing the VTA, RMTg, and DRN was sliced, fixed, and stained for analysis of zif/268 expression using immunofluorescence. Contrary to our predictions, preliminary results indicate that rats receiving 0.5 μg MJN110 avoided the resident rat significantly less than vehicle-treated rats. We expect that decreasing LHb 2-AG will promote active approach behavior under conditions of chronic SDS, which will correlate with increased VTA DA, increased DRN 5HT, and decreased RMTg zif/268 immunofluorescence compared to vehicle. Completion of these studies will contribute to a growing body of evidence suggesting a fundamental role for eCBs in regulating LHb activity and further our understanding of brain circuits/mechanisms that give rise to divergent stress coping strategies.

Disclosures: H.R. Wright: None. **D.E. Ginder:** None. **J.L. Richie:** None. **Z.D.G. Fisher:** None. **A.M. Brown:** None. **R. Urrutia-Carmago:** None. **R.A. Fuchs:** None. **R.J. McLaughlin:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.08

Topic: F.04. Stress and the Brain

Support: Regis University Center for Scholarship and Research Engagement
Nu Rho Psi - Undergraduate Research Grant

Title: Impact of ghrelin signaling on glucagon-like peptide 1 (GLP-1) and prolactin-releasing peptide (PrRP) neural activation and anxiety behavior following cognitive stress in fasted rats

Authors: K. A. COAKLEY, *J. W. MANISCALCO;
Regis Univ., Denver, CO

Abstract: Anxiety-based eating disorders are prevalent, insidious, and particularly devastating. While the anxiolytic properties of fasting may result from a sense of control from regulating food intake, there may also be more basic neurobiological mechanisms by which caloric restriction reduces stress and anxiety. Previous work demonstrates that fasting in rodents leads to reductions in anxiety-like behavior in conjunction with reduced activation of stress-responsive neural circuits, including hindbrain glucagon-like peptide 1 (GLP-1) and prolactin-releasing peptide

(PrRP) neurons. Activation of these neurons contributes to neural, behavioral, and physiological stress responses, and the ability of fasting to reduce activation of these neurons may contribute to behavioral alterations observed in eating disorders. However, it is currently unclear how periods of fasting reduce behavioral anxiety and stress-induced neural activation in the hindbrain. Ghrelin - an orexigenic gut hormone - is released from the gastrointestinal tract during fasting and its signaling can reduce activation of brainstem neurons. Our recent findings indicate that elevated ghrelin levels during fasting are partially responsible for suppressing brainstem neural activation and behavioral responses to systemic cholecystokinin-8, a visceral (bodily) stressor. Considering this, we investigated whether increased ghrelin signaling is also responsible for reducing neural and behavioral responses to cognitive (anticipatory) stressors during fasting. Following an acute overnight fast, rats were administered intraperitoneal injections of either saline vehicle or ghrelin receptor antagonist (GRA). Thirty minutes following injection, rats were placed individually on an illuminated elevated platform for 5 minutes and then sacrificed by transcardial perfusion. Immunohistochemical colocalization of cFos and GLP-1 or PrRP neurons was used to assess neural activation following stress exposure. A separate cohort of overnight fasted rats also received either saline or GRA prior to placement on an elevated plus maze (EPMZ) to assess anxiety-like behavior. Interestingly, we found no differences in cognitive stress-induced activation of GLP-1 or PrRP neurons between the saline and GRA rats. Similarly, we observed no differences in anxiety-like behavior (time spent/entries into the open and closed arms of the EPMZ) between saline and GRA treated rats. Our findings suggest that elevated ghrelin levels are not responsible for reducing anxiety-like behavior and cognitive stress-induced hindbrain neural activation during acute fasting.

Disclosures: K.A. Coakley: None. **J.W. Maniscalco:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.09

Topic: F.04. Stress and the Brain

Support: NIH Grant MH059911

Title: Hindbrain glucagon-like peptide (GLP1) neurons give rise to highly collateralized axonal projections

Authors: *L. RINAMAN[1], H. ZHENG[2];
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Abstract: GLP1 neurons in the caudal nucleus of the solitary tract (cNTS) and intermediate reticular zone (IRt) project widely to CNS regions that regulate autonomic outflow, endocrine

function, food intake, and other motivated behaviors. While the distribution of axons arising from GLP1 neurons is well established in rodents, it is unclear whether GLP1 neurons in the cNTS and IRt target similar brain regions, and whether axons of individual GLP1 neurons collateralize to reach multiple targets. The present study addressed these questions using our newly established Sprague Dawley Cre-driver rat model (i.e., Gcg-Cre). In each rat, two Cre-dependent, retrogradely transported AAVs expressing distinct fluorophors (i.e., AAVrg-EF1a-DIO-hChR2:*mCherry*, or AAVrg-EF1a-DIO-hChR2::*EYFP*) were microinjected stereotaxically into the hypothalamus (PVN) and ipsilateral bed nucleus of stria terminalis (avlBST) to differentially label cell bodies and axon collaterals of GLP1 neurons innervating one or both sites. Rats were perfused 3-4 wk later, and sections processed to enhance red and green fluorophore detection. Axonal inputs to the PVN and avlBST arose from both cNTS and IRt neurons, and large subsets of neurons in both hindbrain locations were double-labeled. In addition, both single- and double-labeled axon collaterals arising from PVN- and avlBST-projecting GLP1 neurons were observed in every medullary, midbrain, diencephalic and telencephalic region known to receive GLP1 axonal input. We conclude that individual GLP1 neurons within the cNTS and IRt project in a reticular-like manner to target widespread CNS regions. This property likely subserves a broad modulatory role of hindbrain GLP1 neurons on widely dispersed neural circuit nodes, and should be kept in mind when using optogenetics or other approaches designed to investigate the role of GLP1 signaling to specific CNS regions.

Disclosures: L. Rinaman: None. **H. Zheng:** None.

Poster

P600. Stress-Modulated Pathways: Brainstem and Other

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P600.10

Topic: G.09. Drugs of Abuse and Addiction

Support: Western University of Health Sciences, Department of Pharmaceutical Sciences

Title: The role of endogenous dynorphin-kappa opioid receptor (DYN-KOR) system in stress-mediated affective disorder and alcohol self-administration in male and female mice

Authors: *S. AHMEDYAR[1], K. LUTFY[2];
[2]Pharmaceut. Sci., [1]Western Univ. of Hlth. Sci., Pomona, CA

Abstract: We investigated the role of endogenous dynorphin-kappa opioid receptor (DYN-KOR) system in the development of stress-mediated affective disorders following a novel repeated variable stress (RVS) exposure model in male and female mice. Subsequently, we assessed the impact of the 10-day RVS model on alcohol self-administration using a free-access one-bottle choice (OBC, 6% EtOH) paradigm in the home cage for 7 weeks. Following a 1-week

withdrawal period, mice were given free-access in a two-bottle choice (TBC) paradigm (6% EtOH vs H_2O then 12% EtOH vs H_2O) for 2 weeks. Next, we assessed for anhedonia with TBC (0.5% sucrose vs water) 48h after a final withdrawal. Naive male (n= 11, 1.6-8.2 months) and female (n=11, 1.6-13.6 months) preprodynorphin (ppDYN) null mice and their male (n=10, 1.6-8.2 months) and female (n=14, 1.6-13.6 months) littermates/controls were used. We also evaluated the effect of weekly administration of KOR antagonist nor-BNI (10 mg/kg) in female C57BL6/J mice (n=9 per treatment/age group, 1.5-12.3 months). Each experiment was repeated 2-3 times and the experimenter was blind to genotype/treatment. Data were mean ± S.E.M. and analyzed by 2-or 3-way ANOVA followed by Fisher's LSD *post-hoc* test with significance set at $p<0.05$. Our results showed no age-related difference in anxiety-like behavior between mice of the two genotypes in both sexes. Old male and female mice lacking ppDYN (KO) exhibited higher immobility than their old wild-type (WT) controls. Immobility time was greater in young WT mice compared to old WT counterparts in both sexes. However, age-related differences were not observed in saline-or nor-BNI-treated C57BL6/J mice nor KO mice. In the OBC, young female ppDYN WT and KO consumed more 6% EtOH than their old controls. There was no difference in EtOH or sucrose consumption/preference between mice of the two age groups. Yet, when young and old female mice of each genotype were combined, female ppDYN KO mice appeared to consume more 6% EtOH compared to WT controls in the OBC but showed less preference for EtOH than WT controls in the TBC. Also, female ppDYN WT mice showed greater sucrose preference than KO mice. In contrast, nor-BNI-treated C57BL6/J mice showed no differences in both EtOH and sucrose intake/preference compared to their saline-treated controls. Together, these results suggest that the DYN-KOR system may be involved in stress-related depressive-like behaviors and subsequent alcohol consumption in female mice. However, these changes could be developing in early age as we did not observe similar changes in nor-BNI-treated mice during adulthood. Studies are underway in male mice of the two genotypes for alcohol consumption.

Disclosures: S. Ahmedyar: None. **K. Lutfy:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045175

Title: Sex-dependent effects of adolescent alcohol and THC co-use on working memory and behavioral flexibility in rats.

Authors: *L. CARRICA, F. WALTER, III, C. CHOI, L. SHI, B. NOONAN, D. TKAC, N.-C. LIANG, J. M. GULLEY;
Univ. of Illinois, Urbana-Campaign, Champaign, IL

Abstract: Previous work in both animal models and humans has demonstrated that exposure to either alcohol or THC, the primary psychoactive component of cannabis, can lead to adverse consequences including deficits in learning, behavioral flexibility, and attention. Additionally, co-use of these substances is relatively common among adolescents and is associated with worse outcomes than the use of either drug alone. In this study, male and female rats were assigned to one of four groups: vehicle/vehicle, 10% ethanol/vehicle, vehicle/THC (3.0-10.0 mg/kg/day), or ethanol/THC. Rats were allowed to self-administer either drug(s) or vehicle from P28-P47. They were subsequently food restricted and underwent training and testing on a lever press, delayed-matching-to-position (DMTP) task from P60-P111. Our hypothesis was that rats who voluntarily consumed both THC and alcohol during adolescence would exhibit reduced accuracy on a working memory task compared to both controls and rats who consumed either drug alone. Our preliminary results suggest that there was an effect of sex as well as a sex-by-treatment interaction on performance on the DMTP task at long response delays, as well as on behavioral flexibility as assessed by a rule shift to a delayed-nonmatching-to-position (DNMTP) task. Females from all exposure groups (except the THC-only rats) outperformed their male counterparts in DMTP testing at long delays, as well as on the DNMTP task. This preliminary analysis suggests that the detrimental effects of THC and alcohol alone, as well as their co-administration, are dependent on sex.

Disclosures: L. Carrica: None. **F. Walter:** None. **C. Choi:** None. **L. Shi:** None. **B. Noonan:** None. **D. Tkac:** None. **N. Liang:** None. **J.M. Gulley:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R21 DA045175

Title: The effects of alcohol and THC co-use during adolescence on synaptic plasticity in the medial prefrontal cortex of Long Evans rats

Authors: *L. SHI[1], S. KANG[2], C. CHOI[3], B. L. NOONAN[3], L. K. CARRICA[3], N.-C. LIANG[4], J. M. GULLEY[5];
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Champaign, IL; [4]Psychology Dept., Univ. of Illinois-Urbana Champaign, Champaign, IL; [5]Psychology Dept., Univ. of Illinois At Urbana-Champaign, Champaign, IL

Abstract: Adolescent exposure to alcohol or delta-9-tetrahydrocannabinol (THC), which is the main psychoactive component in cannabis, has been associated with structural and functional changes in the brain of adult humans and laboratory animals. Considerably less is known about the neurobiological effects of combined exposure to these drugs, which is potentially problematic given that adolescents who use these drugs often consume them together. Here, we addressed this by using male and female Long Evans rats to investigate if adolescent exposure to alcohol-only, THC-only, or combined alcohol-THC would influence synaptic plasticity in the medial prefrontal cortex (mPFC) during adulthood. From postnatal day (P) 30 to P45, rats (n=6/sex/group) voluntarily consumed 0.1% saccharin (SACC) or 10% ethanol in SACC and were injected (s.c.) with vehicle or escalating doses of THC (3.0-20.0 mg/kg). They were sacrificed during young adulthood [P70 to P88] for *in vitro* brain slice recordings (n=4 slices/group) of field EPSPs in layer 5/6 of the prelimbic cortex (PL). Preliminary results suggest high-frequency stimulation (HFS) delivered in layer 2/3 of the PL induced long-term depression (LTD) in controls, but the magnitude of LTD decreased in rats exposed to THC-only or alcohol-only. In alcohol-THC rats, HFS induced a long-term potentiation (LTP)-like response. These preliminary findings suggest that combined use of these two drugs during adolescence may induce enduring changes in mPFC circuitry that are unique to those induced by using either drug alone. We hypothesize that these circuit-level modifications are due to drug-induced alterations in the development of inhibitory signaling in the mPFC.

Disclosures: L. Shi: None. **S. Kang:** None. **C. Choi:** None. **B.L. Noonan:** None. **L.K. Carrica:** None. **N. Liang:** None. **J.M. Gulley:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Fondecyt 1190729
Fondecyt 1200474
Beca ANID 21180866

Title: Perinatal exposure to non-absorbable broad-spectrum antibiotics affects dopamine mesocorticolimbic pathway in a sex-dependent manner in adult rats.

Authors: *C. GONZÁLEZ-ARANCIBIA[1,2,5], V. COLLIO[1,3], F. SILVA-OLIVARES[1,3,6], P. MONTAÑA-COLLAO[1,4], J. MARTÍNEZ-PINTO[1], M. JULIO-PIEPER[5], R. SOTOMAYOR-

ZÁRATE[1], J. A. BRAVO[5];
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Abstract: Bacterial colonization of the infant's gut begins at birth when birth exposes the newborn to a set of bacteria coming from the mother's gut. On the other hand, alterations in gut microbial richness and diversity are associated with several neuropsychiatric disorders, including drug addiction. Preliminary results show that oral administration of a broad-spectrum non-absorbable antibiotic cocktail to a pregnant Sprague-Dawley dam from E18 until P7, lowers gut microbial diversity and richness, while at the same time affecting dopamine receptor expression at the NAcc and VTA in male offspring at P35. Thus, the main aim of this study is to evaluate if early-life exposure to antibiotics (ELEA) impacts drug-seeking behavior, locomotor activity, dopamine receptors levels, and *in vivo* striatal dopamine release in male and female adult offspring of dams treated with antibiotics in the peripartum. Control animals came from dams given only vehicle. ELEA increased preference for methylphenidate (MPH) in the adult male offspring, compared to its control. Interestingly, ELEA females showed a lower preference for MPH than control females. Also, DOPAC levels are lower in both males and females ELEA rats in comparison to controls. Protein analysis revealed changes in the expression of dopamine receptors 1 and 2, and tyrosine hydroxylase throughout the mesocorticolimbic circuit of ELEA rats, in comparison to their respective controls. Finally, female ELEA rats release more striatal dopamine than control females in response to MPH. Altogether, these results suggest that early-life alterations in the development of the microbiota-gut-brain axis affect the mesocorticolimbic structure, and hence, the response to drugs, in adulthood.

Disclosures: C. González-Arancibia: None. **V. Collio:** None. **F. Silva-Olivares:** None. **P. Montaña-Collao:** None. **J. Martínez-Pinto:** None. **M. Julio-Pieper:** None. **R. Sotomayor-Zárate:** None. **J.A. Bravo:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.04

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R21DA048554-02

Title: Transcriptomic adaptations in emotional and sensory brain nuclei in perinatal fentanyl exposed rodents

Authors: *J. OLUSAKIN[1], G. KUMAR[1], C. HAGA[1], M. TURNER[1], M. BASU[1,3], J. ALIPIO[1], C. CALARCO[1], M. FOX[1], A. KELLER[1], S. AMENT[2,3], M. LOBO[1,2];
[1]Anat. and Neurobio., [2]Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD;
[3]Inst. of Genome Sciences, Univ. of Maryland, Baltimore, MD

Abstract: Since the early 2000s, there has been an increase in opioid consumption within the US, including among pregnant women. Use of the synthetic opioid fentanyl has increased ~300% within the last decade. However, little is known about the molecular mechanisms underlying fentanyl use particularly during brain development. Using a rodent model of perinatal fentanyl exposure, we previously demonstrated a lasting emotional and sensory deficit at adolescent and adult ages, consistent with human findings.
In the present study, our aim is to understand the neurobiological mechanisms occurring with developmental fentanyl exposure. We use a Multi-omic sequencing approach to investigate the transcriptional programs dysregulated in fentanyl use in a brain tissue specific manner. Fentanyl was administered in drinking water of pregnant dams from embryonic day 0 through gestational periods until weaning at P21. Tissue punches were collected from 5 brain areas from juvenile aged mice (~P35) and processed for RNA-sequencing. Brain regions analyzed include emotional/reward brain regions: nucleus accumbens (NAc), ventral tegmental area (VTA), prelimbic (PrL) region of the prefrontal cortex and sensory brain regions: primary somatosensory cortex (S1) and ventrobasal (VB) thalamus. These experiments were performed in both sexes as we had observed sex differences in some of the lasting behaviors. We identified differentially expressed gene sets, and performed enrichment analysis, weighted gene coexpression network analysis (WGCNA), and upstream transcription factor analysis. We observed sex driven differences in differentially expressed gene sets within all brain regions analyzed. We identified upstream transcriptional programs and hub genes that could mediate the lasting behavioral changes in fentanyl use. Collectively, our studies are identifying unique gene expression adaptations across multiple brain regions relevant for lasting behaviors observed in perinatal fentanyl exposed mice.

Disclosures: J. Olusakin: None. **G. Kumar:** None. **C. Haga:** None. **M. Turner:** None. **M. Basu:** None. **J. Alipio:** None. **C. Calarco:** None. **M. Fox:** None. **A. Keller:** None. **S. Ament:** None. **M. Lobo:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Methadone elicits a robust transcriptional response during fetal brain development modeled in cortical organoids

Authors: *I. DWIVEDI, D. ZHOU, G. G. HADDAD;
Pediatrics, UC San Diego, La Jolla, CA

Abstract: The recent national opioid epidemic has included a rise in the number of pregnant women seeking treatment for opioid use disorder, which involves the use of methadone, a synthetic opioid analgesic that attenuates withdrawal symptoms. Despite its benefits for mothers, however, fetal exposure to methadone has been shown to induce long-term neuro-cognitive sequelae. Most neurological studies on the effects of methadone exposure have been conducted in murine models or postnatally in humans. Consequently, very little is known about how the drug impacts cellular and molecular human fetal brain development *in utero*. Induced pluripotent stem cell (iPSC)-derived human cortical organoids (hCOs) provide a unique opportunity to assess these aspects of neurodevelopment *in vitro.* Here, we took advantage of hCO technology to probe the molecular underpinnings of methadone's cellular effects on cortical neurogenesis. We conducted bulk mRNA sequencing of 2-month-old hCOs derived from two cell lines that were chronically treated with a clinically relevant dosage of 1 μM methadone. Differential expression analysis (DESeq2) revealed a robust transcriptional response to methadone in hCOs derived from both cell lines, with over 2000 significantly differentially expressed genes common to both ($|FC| \geq 1.5$, p-adj < 0.05). Through pathway enrichment, gene ontology (GO), and network analyses, we observed that chronic treatment with methadone downregulates a hierarchy of 146 extracellular matrix (ECM)-related structural and regulatory genes in hCOs. This includes a key group of genes encoding matricellular proteins (MCPs) and their upstream regulator TGFβ1, which are highly active during development as mediators of ECM structure and degradation. During development, this ECM-regulatory pathway is known to influence cellular proliferation, survival, and migration, as well as neurite outgrowth and synaptogenesis, all of which are associated with the top significantly enriched pathways and GO biological processes in our analyses. Consequently, suppression of this ECM axis may give rise to the cellular phenotypes and ultimate neurobehavioral sequelae associated with fetal methadone exposure. We believe that the results from our study will contribute not only to better informed treatments for pregnant women with opioid use disorders but also to early pharmacological interventions for infants exposed to methadone.

Disclosures: I. Dwivedi: None. **D. Zhou:** None. **G.G. Haddad:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant P60AA011605
CAPES/CNPQ

Title: Naltrexone effects on conditioned approach and orbitofrontal cortex firing activity in adult female rats exposed to binge alcohol in adolescence

Authors: *J. A. POCHAPSKI[1], A. GÓMEZ-A[2], C. A. BOETTIGER[3], C. DA CUNHA[1], D. L. ROBINSON[2];
[1]Biochem., Univ. Federal do Parana, Curitiba, Brazil; [2]Bowles Ctr. for Alcohol Studies, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Psychology & Neurosci., Univ. of North Carolina, Chapel Hill, NC

Abstract: Adolescence is a critical period of brain development, when exposure to addictive drugs can induce long-term impairments to frontolimbic structures, including the orbitofrontal cortex (OFC). In this study, we examined the effects of naltrexone (NTX), an opioid receptor antagonist used for the treatment of alcohol use disorder, on OFC neuronal activity of adult female rats with a history of adolescent intermittent ethanol (AIE) exposure during Pavlovian conditioned approach (PCA). We hypothesized that AIE exposure would promote an enhancement in the OFC response to the conditioned stimulus (CS), and that NTX administration would attenuate this effect. Sprague-Dawley female rats received AIE (5 g/kg intragastric ethanol, 2-day-on/2-days-off) or water (CON) through adolescence (P25-54). In adulthood (~P70) rats began PCA training, where a 30-second CS, presented as a cue light and lever extension, predicted delivery of a 20% sucrose reward. After training, electrode arrays were implanted in the OFC, and electrophysiological recordings were performed during PCA sessions. OFC neuronal activity was evaluated during a PCA baseline session followed by three (counterbalanced) PCA sessions where saline, 1 mg/kg NTX, or 3 mg/kg NTX was administrated (i.p.) 15 minutes prior to the session. Preliminary results demonstrate that NTX administration decreased the number of lever presses and slowed the latency to press in both AIE and CON rats. Conversely, AIE-exposed rats made fewer conditioned receptacle entries than CON-exposed rats, but no robust NTX effect was observed. As we hypothesized, AIE exposure resulted in an enhanced OFC activity in response to CS onset and CS offset events during the PCA baseline session. While NTX effects on the firing rate in response to CS onset and CS offset was minimal, 3 mg/kg NTX robustly decreased the average firing rate of OFC neurons across the whole PCA session only in the AIE-exposed group. These preliminary data demonstrate long-lasting AIE-exposure effects on the OFC processing of conditioned cues and suggests exposure-specific effects of NTX on OFC activity. Future analyses will further determine whether NTX affected behavior and OFC neuronal firing rates in rats with high levels of sign tracking (conditioned approach to the CS) compared to rats engaged in high levels of goal tracking (conditioned approach to the reward receptacle).

Disclosures: J.A. Pochapski: None. **A. Gómez-A:** None. **C.A. Boettiger:** None. **C. Da Cunha:** None. **D.L. Robinson:** None.

Poster

P722. Addictive Drugs: Developmental Effects II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P722.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Miami University Undergraduate Research Award
Miami University Undergraduate Summer Scholars Award
Miami University Honors Program Research Grant

Title: Benzos and Booze: Effects of Adolescent Exposure to Benzodiazepine on Adult Alcohol Consumption

Authors: *Z. PLATOW, M. S. MCMURRAY;
Psychology, Miami Univ., Oxford, OH

Abstract: Benzodiazepines are strong anxiolytic drugs that act at GABA receptors, resulting in overall inhibition of the CNS. Benzodiazepines are one of few drugs that can be both prescribed by a doctor, but also recreationally abused. One in five high schoolers report using benzodiazepines either medically or recreationally. However, the longitudinal effects of benzodiazepine use during adolescence have not been well-investigated. The purpose of this experiment was to determine the effects of benzodiazepine exposure during adolescence on immediate stress levels and later adult alcohol self-administration in rats. Beginning at Postnatal Day (PND) 35, 48 male and female long-evans rats were injected with either 3.2 mg/kg lorazepam (experimental group, N=24) or vehicle (control group, N=24) every other day until PND50. Open Field Test (OFT) and Elevated Zero Maze (EZM) compared stress levels on PNDs 40, 50, and 52 (injection off-days) and PND90. Ethanol self-administration began at PND90 and continued until PND110. At PND110, the animals were given an injection of alcohol 45 minutes before perfusion. Brain tissue was stained with c-fos to evaluate neural activity in the ventral tegmental area and nucleus accumbens. On non-treatment days, benzodiazepine treated rats were shown to have higher adolescent stress levels by both the OFT and EZM results. This difference was greatest at PND52, two days after the final benzodiazepine injection. Preliminary data analysis suggests that these differences in stress persist into adulthood, as shown by the results of the OFT and EZM tests performed on PND90. Additionally, early results regarding ethanol self administration levels indicate that the benzodiazepine-treated rats self administered more ethanol during a 4 hour period than the control rats. Preliminary tissue analysis indicates that the benzodiazepine-treated rats had higher neural activity levels in response to alcohol, which suggests that cross-tolerance was developed due to adolescent benzodiazepine exposure. Based on the results of this study, it appears that adolescent exposure to or abuse of benzodiazepines

causes persisting and long-lasting changes to the brain, ultimately resulting in higher adulthood stress levels and a distorted behavioral response to alcohol.

Disclosures: Z. Platow: None. **M.S. McMurray:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.01

Topic: G.09. Drugs of Abuse and Addiction

Support: CONACyT CB-252348

Title: Alcohol-induced endoplasmic reticulum stress in neuronal cells of the rat hippocampus.

Authors: *K. HERNÁNDEZ-FONSECA, T. SÁNCHEZ-MEDINA;
Neurochemistry, Inst. Nacional De Psiquiatría "Ramón de La Fuente", Mexico city, Mexico

Abstract: Introduction: The Central Nervous System is particularly vulnerable to ethanol-induced damage. They have been observed pathological alterations in brain structure and neuronal function in patients and animal models treated prenatally with alcohol. The cause of ethanol-induced neurodegeneration is unclear; however, it has proposed the participation of endoplasmic reticulum stress in alcohol-induced neuronal damage, such *in vitro* as *in vivo* (Ji, 2012). The endoplasmic reticulum (ER) is essential for synthesizing and modifying proteins, storing and releasing calcium, and promoting lipid biosynthesis and drug detoxification. Dysregulations of the homeostatic state of the ER produce an accumulation of misfolded proteins; under these conditions, the unfolded protein response (UPR) is activated. The UPR has a purpose of restoring homeostasis of the ER, of reducing ER protein load entails the upregulation of the folding machinery along with an expansion of the ER lumen and enhanced degradation of terminally misfolded proteins through ER-associated degradation (ERAD). In addition, UPR activation also causes a temporary shutdown of general translation accompanied by the degradation of a select group of mRNAs encoding secretory proteins and proteins delayed at the translocon, a process known as pre-emptive quality control. Three different ER stress sensors sense the accumulation of unfolded proteins in the ER lumen: two protein kinases, IRE1 (inositol requiring enzyme/endonuclease 1) and PERK (double-stranded RNA-activated protein kinase (PKR)—like ER kinase) and the transcription factor ATF6 (activating transcription factor 6). **Objectives:** To assess whether acute alcohol exposure induces RE stress in the rat brain**.** **Materials and Methods:** Male rats Wistar (200-250 g) were administered intraperitoneally with water (control) or ethanol (3 g/kg, 25%). Furthermore, they were sacrificed 0.5, 1, 2, 4, 8, 12 y 24 hours after treatment. The brains were extracted, dissected, and homogenized, and the expression of ER stress proteins was examined by Western blotting. Results: A significant

increase was observed in the expression of ER stress proteins and activation of the UPR pathway. **Discussion**: Our results show that exposure to ethanol induces a significant increase. The possible cause that triggers this ER stress during alcohol consumption could be the primary metabolite of alcohol degradation, acetaldehyde, which can form protein adducts, induces oxidative stress, and dysregulates calcium homeostasis. **Conclusion:** Acute exposure to ethanol induces ER stress in hippocampal neuronal cells by activating the UPR pathway.

Disclosures: K. Hernández-Fonseca: None. **T. Sánchez-Medina:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant U01AA020926
NIH Grant R01AA012404
NIH Grant U24AA20929
NIH Grant U01AA14095

Title: Transcriptional analysis of the nucleus of the solitary tract after alcohol dependence and stress

Authors: *E. GRANTHAM[1], G. R. TIWARI[2], O. PONOMAREVA[1], M. F. LOPEZ[3], H. C. BECKER[4], R. D. MAYFIELD[5];
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Abstract: Stress exposure contributes to the development of drug and alcohol addiction. Animal models show that stress exacerbates alcohol consumption in alcohol-dependent animals. The nucleus of the solitary tract (NTS) is a critical brainstem region for integrating and relaying peripheral signals to regulate stress responses. To examine the molecular adaptions within this brain region that may contribute to stress-induced alcohol drinking, we exposed animals to chronic intermittent bouts of ethanol vapor (CIE), forced swim stress (FSS), or both (CIE + FSS) and then transcriptionally profiled the NTS at three different timepoint after the last vapor exposure (0-h, 72-h, and 186-h). Using the three timepoints, we were able to identify transient, persistent, and compensatory gene expression patterns related to each of the behavioral treatments. We identified type I interferon signaling as a critical pathway in the development of alcohol dependence. Using likelihood ratio tests, we identified genes that were differentially expressed across time and between groups. Clustering analysis of these genes identified a subset

of genes that fail to normalize in the CIE + FSS group, but not the others. These genes were enriched for cell-to-cell signaling and cellular function pointing to long-term synaptic and structural changes in this brain region because of CIE + FSS. Overall, these results summarize the transcriptomic changes across time in the NTS that may be critical to the development of stress-induced elevations in alcohol consumption and alcohol dependence.

Disclosures: E. Grantham: None. **G.R. Tiwari:** None. **O. Ponomareva:** None. **M.F. Lopez:** None. **H.C. Becker:** None. **R.D. Mayfield:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.03

Topic: G.09. Drugs of Abuse and Addiction

Support: AA027096

Title: Escalation of ethanol intake after innate immune activation by TLR3 agonist, Poly(I:C), in male mice is associated with cell type-specific changes in gene expression

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Abstract: Excessive alcohol (ethanol) consumption is one of the criteria for alcohol use disorder (AUD). AUD is associated with innate immune activation and previous work showed that repeated injections of the TLR3 agonist, Poly(I:C) (PIC) results in immune activation and in escalation of alcohol consumption in C57BL/6J (B6) male mice. We hypothesized that immune activation by PIC causes cell type-specific changes in gene expression in brain reward regions, some of which may drive excessive drinking. We used Every-Other-Day (EOD) ethanol drinking procedure and repeated PIC injections to study the effects of ethanol and immune activation on brain gene expression. B6 male mice were given a total of 9 injections of either saline or 10 mg/kg of PIC every 4 days and were given access to EOD ethanol or water, resulting in 4 groups: saline/water (SW), saline/ethanol (SE), PIC/water (PW), and PIC/ethanol (PE). Brains were harvested 24 hours after the final ethanol session, 6 brain regions (mPFC, Nucleus Accumbens, BNST, Amygdala, Ventral Hippocampus, VTA) were dissected, gene expression was measured using RNA sequencing (RNA-Seq) and compared among the groups. In addition, cell-type specific expression in the mPFC was measured in separate groups of animals using single nucleus RNA-Seq (snRNA-Seq). Repeated PIC injections resulted in escalation of ethanol

consumption in the PE group compared with SE animals. Differential expression analysis of RNA-Seq data identified individual genes (DEGs) and functional groups associated with the effects of innate immune activation, ethanol exposure or both. Compared to the effects of ethanol, immune activation resulted in more drastic changes in gene expression. Specifically, we identified several differentially regulated inflammatory pathways in the PE group versus the SE group across all brain regions. Many genes differentially expressed between the groups were markers of specific cell types (e.g., *Slc17a8* for neurons; *Gfap* for astrocytes; *C1qb* for microglia; *Kdr* for endothelial cells). The snRNA-Seq analysis identified major cell types in mPFC based on cell-specific transcriptional profiles, which included several classes of glutamatergic and GABA-ergic neurons, microglia, oligodendrocytes, endothelial cells, and astrocytes, with many cell-specific genes being regulated by immune activation, ethanol drinking, or both. These cell-specific DEGs and molecular pathways are mechanistic candidates for immune-modulated escalation of ethanol consumption. Supported by R01 AA027096.

Disclosures: B. Kisby: None. **O. Ponomareva:** None. **A. Battenhouse:** None. **Y. Blednov:** None. **R.D. Mayfield:** None. **M.M. McManus:** None. **S. Shanmugam:** None. **R.O. Messing:** None. **R.A. Harris:** None. **I. Ponomarev:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA 3R44AA025804-03S1
University of Kentucky Substance Use Priority Research Area (SUPRA) Super Student Grant Award

Title: Ethanol prevents constitutive decrement of tau phosphorylation immunofluorescence in the cultured neonatal rat hippocampus: Implications for Fetal Alcohol Spectrum Disorders

Authors: *C. BAILEY, J. E. JAGIELO-MILLER, P. KELLER, A. WILCOX, M. A. PRENDERGAST;
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Abstract: Fetal Alcohol Spectrum Disorders (FASDs) are comprised of developmental, behavioral, and cognitive abnormalities caused by prenatal alcohol (EtOH) exposure, affecting an estimated 2%-5% of children and costing $4 billion annually in the United States. While some behavioral therapies are beneficial, the neurochemical mechanisms that underpin FASDs need further elucidation for development of efficacious therapeutics. The role of the tau protein in the hippocampus is especially likely to be involved. Tau catalyzes microtubule polymerization and

maintains structural support for neurons. However, this function is disrupted by hyperphosphorylation (referred to as p-tau). Most of what we know with regard to the cognitive and behavioral ramifications of p-tau comes from neurodegenerative tauopathies, such as Alzheimer's disease. Many of the cognitive deficits observed in neurodegenerative tauopathies overlap to some degree with what is observed in juvenile developmental disabilities, such as FASDs (e.g., selective memory, executive dysfunction, learning difficulties). Thus, the tau protein may be one important mechanism of dysfunction in FASDs, but there is not yet an empirical basis for a tauopathic characterization of FASDs. The purpose of this study is to provide such a basis. We sacrificed 26 Sprague-Dawley rats at postnatal day 10 (3rd trimester human equivalent); organotypic hippocampal slices (N = 242; 111 females) were cultured for 6 days *in vitro* (6DIV; baseline – received no EtOH) prior to any manipulation. We exposed a random 50% of hippocampal slices to 5 days of EtOH (50mM; human equivalent 0.23 BAC) between 6DIV and 11DIV. We placed slices back into regularly replenished media for the remainder of the study. We divided the slices into four groups based on when we visualized and quantified p-tau at the pathological Threonine231 (Thr231) residue. These groups were visualized at 6DIV, 12DIV, 18DIV, and 24DIV. Separate p-tau measures were taken for the subregional hippocampal cornu ammonis 1 (CA1), CA3, genu, and dentate gyrus (DG). No sex differences were detected. EtOH treatment interacted with DIV in the CA1, genu, and DG. In the control group, longer DIV was associated with decreased p-tau fluorescence in the CA1, CA3, genu, and DG. However, in the EtOH-exposed group, the p-tau fluorescence was sustained across DIV in the CA1, genu, and DG. This is the first study to show that EtOH exposure widely prevents tau Thr231 dephosphorylation in the perinatal hippocampus. These findings could lead to innovative pharmacotherapeutic targets for the treatment of cognitive deficits seen in FASDs.

Disclosures: C. Bailey: None. **J.E. Jagielo-Miller:** None. **P. Keller:** None. **A. Wilcox:** None. **M.A. Prendergast:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.05

Topic: G.09. Drugs of Abuse and Addiction

Support: T32 NS105640
R01 DA034696
R01 AA026598
R01 AA027544
F31 AA028726
P30 DA048742

Title: The influence of R7 RGS proteins on inhibitory signaling in VTA dopamine neurons and alcohol consumption

Authors: M. C. DEBAKER[1], T. R. ROSE[2], E. H. MITTEN[1], R. GAO[3], E. MARRON[3], A. M. LEE[3], K. D. WICKMAN[3];
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Abstract: Alcohol use disorder (AUD) is a prevalent medical problem, and the long-term success of current treatments is low, emphasizing the need for new therapeutic approaches. Dopamine (DA) neurons in the ventral tegmental area (VTA) play a critical role in reward and motivation. All drugs of abuse, including alcohol, enhance VTA DA neurotransmission. Increased DA levels can trigger inhibitory G protein-dependent feedback pathways in VTA DA neurons, controlled by $GABA_B$ ($GABA_BR$) and D_2 dopamine (D_2R) receptors, that temper VTA DA neuron output. Alcohol-induced adaptations to these inhibitory feedback pathways may promote development of AUD. Regulator of G protein Signaling (RGS) proteins modulate the strength and sensitivity of G protein-dependent signaling by accelerating G protein deactivation. Members of the R7 subfamily of RGS proteins, which includes RGS6, RGS7, RGS9, and RGS11, have been implicated in drug-related behaviors. Moreover, mice constitutively lacking RGS6 displayed decreased alcohol consumption and reward. The relevant neuron populations underlying this phenotype remain unknown. Using fluorescent *in situ* hybridization, we show that RGS6 and RGS7 are expressed in VTA DA neurons. We used slice electrophysiology to assess the effects of constitutive RGS6 or RGS7 ablation (RGS6KO, RGS7KO) on the strength and sensitivity of $GABA_BR$- and D_2R-dependent signaling. We observed larger somatodendritic currents evoked by the D_2R agonist quinpirole in VTA DA neurons from RGS6KO and RGS7KO mice as compared to wild-type controls ($p=0.005$, n=6-7/sex), but no genotype difference in baclofen-induced currents ($p=0.795$, n=6-7/sex). In a drinking-in-the-dark (DID) paradigm, RGS6KO mice consumed less alcohol compared to wild-type mice ($p=0.0005$, n=11-12/sex), whereas RGS7KO mice consumed similar levels of alcohol compared to wild-type mice ($p=0.682$, n=2-5/sex). To determine whether RGS6 in VTA DA neurons modulates alcohol consumption, we utilized a viral CRISPR/Cas9-mediated ablation approach in VTA DA neurons of adult mice. Guide RNAs (gRNAs) targeting RGS6 or LacZ control were injected into the VTA of DATCre(+):Cas9GFP mice. The impact of viral treatment on D_2R-dependent signaling was assessed electrophysiologically, by measuring quinpirole-induced currents, and behaviorally, using a DID paradigm. Preliminary results indicate that RGS6 gRNA-treated mice consume less alcohol compared to controls ($p=0.0172$, n=8-13/sex), mirroring the phenotype observed in RGS6KO mice. Collectively, our data suggest that RGS6 modulates voluntary alcohol consumption in part via its negative regulation of D_2R-dependent signaling in VTA DA neurons.

Disclosures: M.C. DeBaker: None. **T.R. Rose:** None. **E.H. Mitten:** None. **R. Gao:** None. **E. Marron:** None. **A.M. Lee:** None. **K.D. Wickman:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant R01AA026078
Washington State University, Alcohol & Drug Abuse Program intramural grant

Title: Dreadd and pharmacological inhibition of cerebellar granule cells alter excess alcohol consumption behavior in c57bl/6j mice

Authors: *C. ERIKSON, T. THUET, K. DOUGLAS, D. ROSSI;
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Abstract: Genetic differences in cerebellar sensitivity to alcohol (EtOH) influence EtOH consumption phenotype in animal models, and contribute to risk for developing an alcohol use disorder (AUD) in humans. Recent work from our lab determined that similar to other rodent genotypes, EtOH modulation of cerebellar granule cell (GC) tonic $GABA_AR$ currents is of opposite polarity between C57BL/6 sub-strains, which reflects their respective divergent EtOH consumption phenotypes (ie; B6J: suppression/high EtOH consumption & B6N: enhancement/low EtOH consumption). To assess whether this relationship was causal, we tested whether inhibition of cerebellar GCs in high EtOH consuming B6J mice (using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) and pharmacological techniques) reduces excess EtOH consumption behavior. DREADD "GCGiD" mice were created by crossing B6J Gabra6-Cre mice, which express CRE recombinase under the $GABA_AR$ α6 subunit promoter, with R26-LSL-Gi-DREADD mice from the Jackson Laboratory, thereby ensuring selective hM4Di mutant G protein-coupled receptor expression exclusively in cerebellar GCs. Prior to behavioral experiments, we confirmed, using electrophysiology, that bath applied clozapine-N-oxide (CNO) inhibited cerebellar GCs, and consequently reduced mossy fiber afferent-evoked Purkinje cell output. Interestingly, our data indicate that inhibition of cerebellar GCs by CNO may not be via potassium channel activation but rather enhancement of extrasynaptic $GABA_A$ receptor-mediated tonic current. Contrary to our prediction, GCGiD mice that had been trained in a two-bottle choice, drinking in the dark paradigm (2BC-DID) exhibited a significant increase in the g/kg of a 10% EtOH/10% sucrose mix when CNO was administered i.p. 30-minutes before a 2BC-DID trial. As this was unexpected, we followed up with a pharmacological approach. In voltage-clamped GCs from B6J mice, bath applied Ro15-4513 originally thought to be an α4, α6 $GABA_AR$ inverse agonist, enhanced the GC tonic $GABA_AR$ current and prevented subsequent suppression by EtOH. Accordingly, and in support of our hypothesis, i.p. administered Ro15-4513 (10mg/kg) 5-minutes prior to a 2BC-DID trial significantly reduced the total g/kgs 10% EtOH consumed, licks on the 10% EtOH bottle, and mean BEC achieved. These results highlight the genetically determined divergent response of

GC GABAergic inhibition to EtOH as a possible identifiable risk factor for developing an AUD, and provide an obvious target for pharmaceutical intervention.

Disclosures: C. Erikson: None. **T. Thuet:** None. **K. Douglas:** None. **D. Rossi:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Neuroimmune-mediated alcohol effects on ventral tegmental area microglia and infiltrating leukocytes

Authors: *C. A. SMALL, T. J. CLARKE, J. N. BRUNDAGE, J. D. OBRAY, C. M. MORENO, S. WEBER, J. T. YORGASON, S. C. STEFFENSEN;
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Abstract: Microglia are the primary immune cells in the central nervous system (CNS) and are referred to as its “resident” macrophages. However, their role has recently been shown to extend far beyond immunity. The effects of ethanol on the brain are closely linked to neuroimmune responses, mediated by microglia that are present in the healthy brain from the time of development. Normally, the blood-brain barrier (BBB) prevents the infiltration of cells and foreign pathogens from crossing from the periphery into the CNS. However, peripheral monocytes are known to infiltrate the CNS in response to seizures, traumatic brain injury, infection, and multiple sclerosis. The aim of this study was to determine the effect of acute ethanol (EtOH) on microglia activation and monocyte infiltration into the CNS. We hypothesized that acute EtOH would lead to an increase in neuroinflammation by activating “resident” microglia to an inflammatory polarization (M1) and induce the infiltration of macrophages across the BBB. Using the Macrophage FAS-Induced Apoptosis (MaFIA) mouse model (GFP+ on Csf1r promoter), fluorescent microscopy, and flow cytometry we assessed the presence and phenotype of microglia and infiltrating macrophages following 1, 2, and 4 g/kg ethanol at 0.5, 1, and 2 hours post-injection. By measuring volume/surface area of microglia in the ventral tegmental area (VTA) and nucleus accumbens (NAc) following EtOH, we found that EtOH enhanced the number of macrophages in the blood, caused microglia activation in the VTA and NAc, and that microglia shifted toward an M1 polarization. However, some of our findings were counter to our hypothesis. We found that EtOH decreases the number of infiltrating monocytes in the VTA and NAc. It is possible that other cells like T and B cells are

recruited across the BBB. In addition, depleting macrophages in the blood approximately 50% with liposomal clodronate reduce the inhibitory effects of EtOH on VTA GABA neuron firing rate vs clodronate control liposomes at 24 hours post injection. These findings suggest a neuroimmune connection for acute EtOH use and challenge the dogma that EtOH has exclusively central effects on dopaminergic neuronal activity and release. Studies are ongoing to determine the effects of macrophage depletion on EtOH enhancement of DA release and behavioral effects. Keywords: alcohol, microglia, macrophage, inflammation, cytokines, neuroimmune

Disclosures: C.A. Small: None. **T.J. Clarke:** None. **J.N. Brundage:** None. **J.D. Obray:** None. **C.M. Moreno:** None. **S. Weber:** None. **J.T. Yorgason:** None. **S.C. Steffensen:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.08

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA025996

Title: Proteomic analysis of central amygdala systems regulated by mifepristone in the context of alcohol dependence

Authors: *L. MONTEAGUDO-ROMERO, K. N. EDWARDS, J. J. GUIDRY, S. EDWARDS;
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Abstract: Alcohol use disorder (AUD) is a chronic psychiatric disorder characterized by escalated alcohol use and the emergence of negative emotional states during withdrawal. Studies have shown a dysregulation of glucocorticoid signaling in AUD, both at the level of the HPA axis and within specific brain areas such as the central amygdala (CeA). Excessive activation of brain GRs is hypothesized to promote cognitive dysfunction, negative emotional states, and escalated drinking, all common features of AUD. Previous work in our lab has demonstrated an increase in GR phosphorylation in the CeA during alcohol withdrawal in dependent animals. Importantly, the GR antagonist mifepristone reduces escalated alcohol drinking in both rodents and humans. Investigation of functional neuroadaptations in CeA protein networks in the context of alcohol dependence and mifepristone therapy is crucial to our understanding of these conditions. The main aims of this study were to first determine differential CeA protein expression profiles in alcohol dependence and then to examine which of these are “normalized” by mifepristone treatment. Male Wistar rats underwent 10 weeks of exposure to either chronic intermittent ethanol vapor (CIEV) or air (control condition). In week 7, half of the animals

received subcutaneous placebo pellets and the other half received mifepristone pellets (21-day slow release). Three weeks after, rats were sacrificed during acute (6hr) withdrawal from CIEV (and identical time in controls) and the CeA was taken for proteomics analysis. Ingenuity pathway analysis revealed that out of 3050 proteins detected in the CeA, 253 were altered by alcohol (but not mifepristone), whereas an additional 142 were altered by alcohol and "normalized" by mifepristone. Several of these in the latter category represent "druggable" targets and novel treatment avenues for AUD that will be further investigated in future behavioral studies.

Disclosures: L. Monteagudo-Romero: None. **K.N. Edwards:** None. **J.J. Guidry:** None. **S. Edwards:** None.

Poster

P742. Alcohol: Cell Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P742.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NADIA: UO1 AA 028710
T32 AA 025606
DEARC: P50 AA017823

Title: Loss of cholinergic phenotype in basal forebrain neurons following adolescent intermittent ethanol exposure is mediated by the p75 neurotrophin receptor

Authors: *B. T. KIPP[1], L. M. SAVAGE, Ph.D.[2];
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Abstract: Basal forebrain cholinergic neurons enter a period of protracted quiescence following adolescent intermittent ethanol exposure with significant reductions in the expression of choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (vAChT), however the mechanism through which this occurs is unknown. To date, activity at the p75 neurotrophin receptor (p75NTR) appears to drive the suppression of the neuronal basal forebrain cholinergic phenotype in multiple disease states, but p75NTR has not been examined in relation to cholinergic cell pathology in ethanol models. We administered a novel p75NTR modulator, LM11A-31, during ethanol exposure to determine if cholinergic neurons could be protected from developmental ethanol toxicity. Male and female Sprague Dawley rats underwent a adolescent intermittent ethanol exposure (AIE) which mimics the binge withdrawal ethanol consummatory pattern of alcohol drinking typically observed in adolescents. Rats received oral gavages of 5g/kg of 20% ethanol from post-natal day 25-45 with and without LM11A-31, or volume matched

water. Western blot data suggests that modulating the p75NTR during ethanol exposure protects ChAT expression in the nucleus basalis magnocellularis, and vAChT expression in the prefrontal cortex. Additionally, it appears that LM11A-31 administration during ethanol exposure prevents the loss of hippocampal neurogenesis as measured through doublecortin. Based on this preliminary data, we will measure changes in neurotrophin and proneurotrophin expression at cholinergic basal forebrain projection sites following AIE during the final ethanol intoxication, acute withdrawal, and protracted withdrawal to identify potential p75-ligand interactions that are responsible for driving the suppression of neuronal cholinergic phenotype in development models of ethanol exposure.

Disclosures: B.T. Kipp: None. **L.M. Savage:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.01

Topic: H.07. Long-Term Memory

Support: NIA F32 (AG071209)
NIA (AG050787)
NIA (AG051807)
NIA (AG054349)
NIA (AG054349)
NIMH (MH101491)
NIDA (DA025922)

Title: Exercise opens a 'molecular memory window' to facilitate changes in gene expression, synaptic plasticity and memory

Authors: *A. A. KEISER[1], T. DONG[1], E. A. KRAMÁR[1], C. BUTLER[2], D. P. MATHEOS[1], L. TONG[2], N. C. BERCHTOLD[2], S. CHEN[3], M. SAMAD[3], C. MAGNAN[3], J. BEARDWOOD[1], S. SHANUR[1], A. RODRIGUEZ[1], P. BALDI[3], C. W. COTMAN[2], M. A. WOOD[1];
[1]Neurobio. & Behavior, [2]Neurol., [3]Inst. for Genomics and Bioinformatics, Univ. of California, Irvine, Irvine, CA

Abstract: We have previously demonstrated that exercise enables hippocampal-dependent learning in conditions that are normally subthreshold for encoding and memory formation and depends on hippocampal induction of brain-derived neurotrophic factor (BDNF) as a key mechanism. In male mice with prior exercise experience, only a brief exercise period is required to reactivate the increase of BDNF in the hippocampus, suggesting that there exists a 'molecular memory' for the prior exercise experience that facilitates subsequent learning. Here, we find that

brief 2-day re-introduction to exercise following a sedentary delay was sufficient to re-gain cognitive benefits in object location memory and enhance hippocampal LTP (similar to effects of initial exercise) relative to controls. We hypothesize that an epigenetic molecular memory of exercise as a previous experience primes specific genes for subsequent activation upon new learning, resulting in facilitated memory formation. RNA-Sequencing data from dorsal hippocampus tissue taken from male mice during the memory consolidation window, post-exercise, demonstrates that different exercise parameters led to distinct transcriptional profiles. Data point to a novel plasticity protein, ACVR1C as being up-regulated in conditions in which exercise facilitates learning and maintained throughout the 'molecular memory window' for exercise. Using intra-hippocampal delivery of AAV1-ACVR1C point mutant constructs, we find that ACVR1C bi-directionally modulates hippocampus-dependent learning and memory as observed by either disrupted or facilitated performance in object location memory and context fear conditioning. Using slices from the same mice, we also find bi-directional modulation of hippocampal LTP. Together, these data point to ACVR1C as critical in learning and memory and as a potential key contributor in the maintenance of the 'molecular memory window' for exercise.

Disclosures: A.A. Keiser: None. **T. Dong:** None. **E.A. Kramár:** None. **C. Butler:** None. **D.P. Matheos:** None. **L. Tong:** None. **N.C. Berchtold:** None. **S. Chen:** None. **M. Samad:** None. **C. Magnan:** None. **J. Beardwood:** None. **S. Shanur:** None. **A. Rodriguez:** None. **P. Baldi:** None. **C.W. Cotman:** None. **M.A. Wood:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.02

Topic: H.07. Long-Term Memory

Support: CAPES
CNPq

Title: GluN2B-containing NMDA receptors in the dorsal hippocampus are involved in memory labilization and reconsolidation effects of delta-9 tetrahydrocannabinol

Authors: *A. M. RAYMUNDI, J. M. B. SOHN, B. W. SALEMME, C. A. J. STERN;
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Abstract: Labilization is a boundary condition to memory retrieval-induced reconsolidation. Evidence suggests that GluN2B and GluN2A-containing NMDA and cannabinoid type-1 (CB1) receptors located in the dorsal hippocampus (DH) may contribute to these processes. Delta-9-tetrahydrocannabinol (THC) is a partial agonist of CB1 receptors, however, its effects on

labilization are unexplored. The present study aimed to investigate whether and how THC affects fear memory labilization and reconsolidation. Male Wistar rats with bilateral cannulas implanted to the DH were conditioned to Context A. 20 min before a brief memory retrieval, rats were treated with THC i.p. (0.002 or 0.3 mg/kg) or vehicle (VEH) and, immediately after, received VEH or anisomycin (ANI, protein synthesis inhibitor) into the DH. In experiment 2, 20 min before retrieval, rats received VEH or THC 0.3 i.p. and VEH, PEAQX (GluN2A-NMDA receptor antagonist), or ifenprodil (IFE; GluN2B-NMDA receptor antagonist) into the DH. Immediately after retrieval, rats received VEH or ANI. In the third experiment, 20 min before retrieval, rats received VEH or THC 0.002 i.p. and VEH, PEAQX, or IFE into the DH. 1 and 7 days later the animals were exposed to Test A_1 and Test A_2 to evaluate the drug effects. Repeated-measures ANOVA followed by Newman-Keuls post hoc test was used. Results were expressed as mean ± SEM of the percentual freezing time. All procedures were approved by the local ethics committee #1247. The infusion of ANI into the DH (Test A_1: 32.4 ± 5.6; Test A_2: 23.7 ± 5.1) or the treatment with THC 0.002 (Test A_1: 42.6 ± 6.0; Test A_2: 23.7 ± 5.2) before memory retrieval significantly reduced the freezing behavior during Test A_1 and Test A_2 when compared to controls (Test A_1: 74.1 ± 4.7; Test A_2: 52.4 ± 8.1), suggesting an impairment in memory reconsolidation. The pretreatment with THC 0.3 abolished the ANI-induced effect (Test A_1: 68.5 ± 6.1) when compared to control (Test A_1: 74.1 ± 4.7), suggesting an impairment in memory labilization. This effect was restored by the blockade of GluN2B in the DH (Test A_1: 15.6 ± 4.3), but not GluN2A (Test A_1: 60.8 ± 5.4), when compared to control (Test A_1: 69.5 ± 4.8). The blockade of GluN2B, but not GluN2A, impaired the THC 0.002 (Test A_1: 74.3 ± 5.4) effect in memory reconsolidation when compared to control (Test A_1: 68.7 ± 5.0). Our findings indicate a bidirectional effect of THC, whereas an ultra-low dose impaired memory reconsolidation, a higher dose hindered its labilization. These effects depended on hippocampal GluN2B-NMDA receptors, suggesting that activation of CB1 receptors may modulate the contribution of hippocampal GluN2B subunits to memory labilization and reconsolidation.

Disclosures: A.M. Raymundi: None. **J.M.B. Sohn:** None. **B.W. Salemme:** None. **C.A.J. Stern:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.03

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant
NSERC CGSD

Title: Destabilization of strongly encoded spatial memories in mice requires muscarinic cholinergic receptor activation

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Abstract: Previously consolidated memories can become destabilized following exposure to reminder cues associated with the original learning experience. This renders the memory labile and vulnerable to modification. This updating can include strengthening, weakening, or integrating new information into the existing memory. A destabilized memory must then be reconsolidated to persist in long-term memory. Stronger or older memories can be destabilization-resistant, but exposure to salient novelty during memory reactivation can render them labile. Using rats, we have previously shown that object and spatial memory destabilization requires acetylcholine acting at muscarinic cholinergic receptors (mAChRs) within perirhinal cortex and the dorsal hippocampus, respectively. Here, we utilized the hippocampus-dependent object location task to demonstrate that 1) NMDA receptor antagonism with MK-801 (0.1 mg/kg, ip) prevents spatial memory reconsolidation in male C57BL/6 mice; 2) strongly encoded object location memories resist destabilization, but exposure to novelty during reactivation renders them labile and vulnerable to weakening; 3) non-specific mAChR antagonism with scopolamine (0.3 mg/kg, ip) prevents both standard and novelty-induced destabilization of object location memories; and 4) activation of M_1 mAChRs during memory reactivation mimics the effect of novelty, facilitating destabilization of an otherwise-resistant object location memory in the absence of salient novelty during reactivation. This research provides converging evidence to support the idea that acetylcholine acting at mAChRs is critical for destabilization of different types of otherwise-resistant memories, which could be a general mechanism for this process. In addition, this research broadens our understanding of long-term memory storage dynamics and suggests implications for the understanding and treatment of disorders characterized by inflexible memories, such as Alzheimer's Disease and PTSD.

Disclosures: A.E. Huff: None. **W.S. Messer:** None. **B.D. Winters:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.04

Topic: H.07. Long-Term Memory

Support: NSERC Grant 400176

Title: Age-related object memory modification impairments can be ameliorated by systemic M1 muscarinic acetylcholine receptor activation

Authors: *K. H. JARDINE, S. D. MCGRAW, H. EDWARDS, B. D. WINTERS;
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Abstract: Memory reactivation opens the door for memory trace updating. Upon reactivation, synaptic connections within the memory trace can destabilize and remodel for a brief period before being re-consolidated. In some cases, however, memories show resistance to modification. For example, memory updating deficits are commonly reported among older individuals, especially for navigational information. There is currently no treatment for these memory updating deficits, potentially due to a limited understanding of neural factors facilitating reactivation-based declarative memory updating. Our lab suspects that the cholinergic system has the unique potential to promote the updating of inflexible memories. In support of this, we previously found that muscarinic acetylcholine (ACh) receptor (mAChR) signalling at the M1 subtype within perirhinal cortex (PRh) is critical for object memory destabilization and reactivation-based object memory updating. Also, ACh apparently supports the prediction error signal for non-affective information, and an element of prediction error during memory reactivation seems to reliably induce destabilization. Correspondingly, in aging and memory research, there is a well-established decline in cholinergic transmission and reduced cortical mAChR levels in the aging brain, which could explain age-related memory inflexibility. Considering these findings, we hypothesize that age-related cholinergic system dysfunction underlies memory updating deficits experienced by elderly individuals; thus, promoting mAChR signalling during memory reactivation could restore these deficits. First, to explore this hypothesis, we needed to characterize declarative memory updating performance in mice at different ages. We used the post-reactivation object memory modification (PROMM) task for mice—where a reactivated object memory is updated with new contextual information—to measure object memory updating in 3-, 6-, and 12-month-old mice. We found that 3-month-old mice showed intact PROMM task performance, 6-month-old mice showed a mild impairment, while 12-month-old mice seemed severely impaired. Next, we assessed whether activating M1-mAChRs during reactivation could restore object memory updating in the 12-month-old group. To test this, we administered the M1-selective agonist CDD-0102A prior to object memory reactivation in the PROMM task in 12-month-old mice. Remarkably, pre-reactivation systemic M1-mAChR activation enhanced PROMM task performance of 12-month-old mice. Here we showed a promising strategy for promoting the maintenance of memory accuracy as we age.

Disclosures: K.H. Jardine: None. **S.D. McGraw:** None. **H. Edwards:** None. **B.D. Winters:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.05

Topic: H.07. Long-Term Memory

Support: CAPES/NUFFIC

Title: The activity of phosphodiesterases 4, 4B and 4D in the dorsal hippocampus contributes to sustaining the fear memory after retrieval

Authors: *J. M. B. SOHN[1], N. C. CARDOSO[1], J. PRICKAERTS[2], C. A. J. STERN[1];
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Abstract: Upon retrieval, a consolidated memory can become destabilized and subsequently restabilized by the reconsolidation process. Modulation of 3´, 5´cAMP signaling by inhibiting phosphodiesterase 4 (PDE4) regulates protein kinase A (PKA) activity and has emerged as a potential target for improving memory consolidation and extinction persistence. However, the role of PDE4 in memory reconsolidation remains unexplored. The aim of the present study was to investigate the role of PDE4 and its subtypes PDE4B and 4D in fear memory reconsolidation. Adult male Wistar rats with bilateral cannulas implanted to the dorsal hippocampus (DH) underwent contextual fear conditioning to Context A. After a brief memory retrieval, roflumilast (unselective PDE4 inhibitor; ROF 9 ng/0.5 µl/side), A33 (PDE4B inhibitor; 1 µM/0.5 µl/side), Gebr-32A (PDE4D inhibitor; 1 µM/0.5 µl/side) or vehicle (VEH) were injected into the DH. One and 10 days later the animals were exposed to Test A_1 (TA_1) and Test A_2 (TA_2), respectively, to evaluate the drug effects. In experiment 2, animals were treated with ROF, A33, Gebr-32A, or VEH 5 min after a brief memory retrieval. TA_1 was omitted and the animals were exposed only to TA_2 10 days later. In experiment 3, animals were treated with ROF or VEH 5 min after a brief memory retrieval. 5 min after TA_1, animals received VEH or anisomycin (ANI; protein synthesis inhibitor; 20 µg/side) and were exposed to TA_2. All experiments were approved by the local ethics committee. The results are expressed as mean ± S.E.M. Animals treated with ROF (20.9 ± 6.1), Gebr-32A (13.4 ± 4.7) or A33 (17.4 ± 1.7) presented significantly less freezing behavior than controls (53.0 ± 6.0) during TA_2, but not during TA_1. When TA_1 was omitted, animals treated with ROF (41.1 ± 10.1), Gebr-32A (40.5 ± 11.3) or A33 (42.3 ± 8.8) behaved similarly to controls (35.3 ± 12.8) during TA_2, suggesting that TA_1 exposure is necessary for PDEs inhibition effect. Animals treated with ROF after retrieval and ANI immediately after TA_1 did not show significantly different levels of freezing during TA_2 (ROF-ANI: 29.0 ± 5.2) when compared to controls (53.3 ± 6.7), suggesting that protein synthesis during TA_1 is partially involved in PDEs inhibition effect. No difference among VEH-ANI-treated group (31.5 ± 5.8) when compared to controls (53.3 ± 6.7) was observed. The results suggest that upon retrieval, the hippocampal activity of PDE4, mainly subtypes 4B and 4D, is not required for fear memory reconsolidation, but is involved in sustaining fear memory in a long-term manner. The present results suggest that behavioral tagging mechanisms might underlie the effects of PDE4, 4B, and 4D inhibition.

Disclosures: J.M.B. Sohn: None. **N.C. Cardoso:** None. **J. Prickaerts:** Other; JP has a proprietary interest in the PDE4 inhibitor roflumilast. **C.A.J. Stern:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.06

Topic: H.07. Long-Term Memory

Title: Phosphorylation state of histone deacetylase 3 (HDAC3) modulates long-term memory formation and synaptic plasticity

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Abstract: Histone deacetylase 3 (HDAC3) negatively regulates long-term memory in the hippocampus by inhibiting transcription of genes important for learning and memory. While several studies have shown that inhibiting or deleting HDAC3 in hippocampal neurons facilitates memory formation, the mechanisms regulating HDAC3 activity are less understood. However, previous studies from our lab suggest that the phosphorylation state of HDAC3 modulates its enzymatic activity and consequently, may be important in regulating long-term memory. We previously found in a collaborative study that the expression of point mutant constructs mimicking phosphorylation of HDAC3 in cultured dorsal root ganglion cells negatively regulates spinal cord recovery after injury. Unpublished data from our lab also revealed that HDAC3 phosphorylation levels were elevated in hippocampal neurons of aging (18-month-old) mice which correlated with impaired performance on an object location memory task in comparison to young mice. Taken together, these findings lead us to hypothesize that phosphorylation of HDAC3 likely negatively regulates hippocampal-dependent memories. To test this, we developed an HDAC3 phosphomimetic mutant virus to study the function of phosphorylated HDAC3 on hippocampal-dependent memory formation. Mice received infusions of either the HDAC3 phosphomimetic virus or an empty vector virus into the dorsal hippocampus. Then, we tested mice on an object location memory (OLM) task before measuring synaptic plasticity with slice electrophysiology in CA1. Mice expressing the HDAC3 phosphomimetic mutant in the dorsal hippocampus had impaired OLM performance and synaptic plasticity in CA1 in comparison to mice that received the empty vector virus. Overall, our findings indicate that phosphorylated HDAC3 disrupts hippocampal-dependent memory formation and synaptic plasticity. It’s possible that increased levels of phosphorylated HDAC3 in the hippocampus could contribute to memory impairments associated with aging.

Disclosures: A.C. Rodriguez: None. **A.A. Keiser:** None. **E.A. Kramár:** None. **T. Dong:** None. **J.L. Kwapis:** None. **D.P. Matheos:** None. **M.A. Wood:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.07

Topic: H.07. Long-Term Memory

Support: NIH-NINDS Child Neurologist Career Development program K12NS098482

Title: Histone post-translational modifications underlying the persistent effects of early-life exercise on hippocampal memory

Authors: *A. M. RAUS[1], S. YANG[4], T. FULLER[2], N. E. NELSON[3], S. D. A. VALIENTES[2], E. BASSEY[5], A. S. IVY[2];
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Abstract: Introduction: Early-life experiences such as exercise can influence memory function throughout life. Understanding the molecular mechanisms by which early-life exercise (ELE) influences memory can lead to novel interventions for ameliorating disorders of memory function. Our lab recently demonstrated that exercised juvenile rodents exhibited improved hippocampal long-term memory and basal synaptic physiology that lasted into adolescence (Ivy et al., *Sci Rep* 2020). Epigenetic mechanisms, such as histone modifications, are strong candidates underlying this phenomenon: they can bridge environmental experiences with gene expression, can be persistent or transient, and can have lasting influences on neuronal function. We hypothesize that exercise in early life facilitates enabled memory formation in adolescence by promoting stable epigenetic modifications at neuroplasticity-associated genes. Methods: We crossed a mouse line expressing *loxP*-flanked Gt(ROSA)26Sor$^{tm1(CAG\text{-}birA,\text{-}mCherry/Rangap1,\text{-}EGFP/Rpl10a)Evdr}$ (known as NuTRAP) with mice expressing Emx1$^{IRES\ cre}$ for evaluating transcriptional profiles and histone modifications (H4K8ac, H3K27me3) from isolated hippocampal neurons expressing the NuTRAP cassette. “Emx-NuTRAP” mice were either sedentary or underwent three weeks of voluntary running (postnatal days (p)21-41). Hippocampal tissue was collected on p42. Samples were processed using the INTACT (isolation of nuclei tagged in specific cell types) and TRAP (translating ribosome affinity purification) techniques from the same single cell suspension, followed by CUT&RUN-seq and RNA-seq . RT-qPCR and RNA-seq was also performed on wild-type mice that underwent ELE (for either 1 or 3 weeks) followed by object location memory training (Ivy et al., *Sci Rep* 2020). RT-qPCR and ChIP-qPCR for H4K8ac was performed from hippocampi of wild type mice after ELE alone. Results: After 3 weeks of ELE (p21-41), Emx-NuTRAP mice showed increased memory-

associated gene expression, including BDNF. CUT&RUN-seq data to assess H4K8ac and H3K27me3 occupancy is currently being analyzed. 1-week of ELE (p21-27) was sufficient to increase H4K8ac occupancy at the NR4A1 promoter when evaluated at p42, but insufficient to produce persistent altered NR4A1 gene expression. Preliminary RNA-seq on dorsal hippocampus from wild-type mice after a sub-threshold learning event indicates increased expression of memory-associated genes. Conclusions: Early-life exercise alters histone post-translational modification occupancy, potentially providing a mechanism for priming memory-associated gene expression after a sub-threshold learning event.

Disclosures: A.M. Raus: None. **S. Yang:** None. **T. Fuller:** None. **N.E. Nelson:** None. **S.D.A. Valientes:** None. **E. Bassey:** None. **A.S. Ivy:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.08

Topic: H.07. Long-Term Memory

Support: 1R01MH119541-01A1
5R21DA039417-02
5R21DA041547-02

Title: Spatial-temporal regulation of a novel lncRNA

Authors: I. ESPADAS, E. GRINMAN, J. WINGFIELD, B. RAVEENDRA, ***S. PUTHANVEETTIL**;
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Abstract: Specific changes in the gene transcription are a pre-requisite for long-term memory (LTM) storage. However, it remains largely unknown whether transcriptional changes in long noncoding RNAs (lncRNAs) are associated with learning and whether these changes mediate the consolidation of long-term memories (LTM). Here we report the identification of lncRNA D17Rik from the unbiased analysis of gene expression changes in dorsal CA1 neurons of mouse hippocampus following contextual fear conditioning (CFC). D17Rik expression is upregulated specifically in CA1 neurons within an hour of CFC training. We find that the knock-down (KD) of D17Rik in CA1 does not affect acquisition, recall or extinction of CFC memory, but impairs consolidation of CFC memory. Importantly D17Rik KD in CA3 neuron does not affect consolidation suggesting a specific role for D17Rik in CA1 neurons. Our study illuminates the significance of spatial-temporal regulation and function of D17rik lncRNA in LTM.

Disclosures: I. Espadas: None. **E. Grinman:** None. **J. Wingfield:** None. **B. Raveendra:** None. **S. Puthanveettil:** None.

Poster

P846. Consolidation and Reconsolidation: Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P846.09

Topic: H.07. Long-Term Memory

Support: PIRE

Title: Role of DNA recombination in long term memory formation in honey bee

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Abstract: Over days, bee colonies trade changing floral resources through plastic behaviour of foragers. Foragers may,meticulously, decide to visit a particular flower because they know the visit is worth. This mechanism of selection based on learning, may help us understand the process of Long term memory (LTM) formation and consolidation. Arabinoside cytosin (ARA-C), as an antimetabolite drugs, is known to inhibit DNA synthesis. The process of memory formation depends on the transcription and translation of DNA, but the role of DNA recombination in memory stabilization is unknown. Here, we asked if inhibition of DNA recombination affect LTM formation. In this study we used ARA-C to block the DNA recombination process in honey bee. Previous studies have shown that ARA-C causes a progressive decreaseof cognitive activity in patients treated for breast cancer. Considering this drug's side effect, we hypothesized that ARA-C would block the LTM formation without affecting affecting the learning process. We analyzed the STM formation 20 minutes after completing the conditioning and the LTM 24 hours after, obtaining no significant differences in the proportion of individuals exhibiting STM. However, we found a significant difference in the LTM and no LTM expression between both groups. We discuss these results of the role of the DNA recombination in the LTM consolidation.

Disclosures: F. Noel: None. **L. Sanchez:** None. **A. Mehmet:** None. **M. Giurfa:** None. **T. Giray:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.01

Topic: H.09. Spatial Navigation

Support: NIMH Grant MH119391
NINDS Grant NS094094

Title: A selective role for the mPFC during choice & deliberation, but not spatial memory retention over short delays.

Authors: *K. S. KIDDER[1], J. T. MILES[1], P. M. BAKER[3], V. I. HONES[1], D. H. GIRE[2], S. J. MIZUMORI[1];
[2]Psychology, [1]Univ. of Washington, Seattle, WA; [3]Psychology, Seattle Pacific Univ., Seattle, WA

Abstract: Past research reveals that the medial prefrontal cortex (mPFC) and hippocampus (HPC) are both structures vital to spatial-working memory and decision-making. Recent evidence suggests that interactions between these two structures are dynamic and task dependent. However, there exists uncertainty surrounding the specific conditions that recruit the mPFC during these tasks, specifically regarding its role in retaining spatial information in memory during delay periods. To address this issue, we tested rats on a spatial-delayed alternation (SDA) task in which we utilized a closed-loop optogenetic system to transiently disrupt mPFC activity during different task epochs (delay, choice, return). By analyzing the effects of mPFC disruption on choice accuracy and a deliberative behavior known as vicarious-trial-and-error (VTE), our study revealed several interesting findings regarding the role of the mPFC in spatial-working memory tasks. The main findings include: (1) choice accuracy in the SDA task was impaired when the mPFC was disrupted during the choice epoch and was not impaired when disruption was applied to delay or return epochs. (2) mPFC disruption resulted in a non-epoch specific reduction in VTE occurrence, however there was a strong trend towards less VTEs occurring after choice epoch disruption. (3) The decrease in choice accuracy following mPFC disruption was significantly correlated with a decreased occurrence of VTE behaviors. Taken together, findings from this study suggest that, during spatial decision-making, contributions made by the mPFC are specific to points of deliberation and choice (not delay), and that VTEs are a deliberative behavior which relies on intact mPFC function.

Disclosures: K.S. Kidder: None. **J.T. Miles:** None. **P.M. Baker:** None. **V.I. Hones:** None. **D.H. Gire:** None. **S.J. Mizumori:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.02

Topic: H.03. Decision Making

Support: NIH Grant T32NS099578
NIMH Grant MH119391

Title: Vicarious trial and error classification using trajectories and hippocampal oscillations

Authors: *J. T. MILES, K. S. KIDDER, Z. WANG, Y. ZHU, D. H. GIRE, S. J. Y. MIZUMORI;
Univ. of Washington, Seattle, WA

Abstract: Vicarious trial and error behaviors (VTEs) indicate periods of indecision during decision-making, and have been proposed as a behavioral marker of deliberation. In order to understand the neural underpinnings of these decision-making variants, researchers need the ability to readily and confidently distinguish VTEs from non-VTEs. Here we utilize a small set of trajectory-based features and standard machine learning classifiers to identify VTEs from non-VTEs for rats performing a spatial delayed alternation task (SDA) on an elevated plus maze. We also show that previously reported features of the hippocampal field potential oscillation can be used in the same types of classifiers to separate VTEs from non-VTEs with above chance performance. However, we caution that the modest classifier success using hippocampal population dynamics does not identify many trials where VTEs occur, and show that combining oscillation-based features with trajectory-based features does not improve classifier performance compared to trajectory-based features alone. Overall, we propose a standard set of features useful for trajectory-based VTE classification in binary decision tasks, and support previous suggestions that VTEs engage a network including, but likely extending beyond, the hippocampus.

Disclosures: J.T. Miles: None. **K.S. Kidder:** None. **Z. Wang:** None. **Y. Zhu:** None. **D.H. Gire:** None. **S.J.Y. Mizumori:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.03

Topic: H.09. Spatial Navigation

Support: Funding: NIDCD R01-DC014367, DARPA HR0011-18-2-0024 to LMK

Title: Coordinated respiratory and brain rhythms during spatial navigation and learning

Authors: *A. SHERIFF, L. M. KAY;
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Abstract: Several recent studies have demonstrated a role for the respiratory rhythm in widespread oscillatory coherence throughout the limbic system and neocortex, similar to other global rhythms. The nasal respiratory rhythm, which produces robust olfactory bulb (OB) oscillations, has especially been related to episodic memory, locomotion, and exploration. The piriform cortex (PC) is implicated in propagating the OB driven respiratory rhythm, however this region has not been studied along with both hippocampal theta and nasal respiratory rhythm during exploratory behaviors. Here, we characterize dynamic respiratory and theta network configurations associated with the perceptual environment during spatial navigation and learning. In the first set of experiments, local field potentials (LFPs) were recorded in olfactory areas including the OB and PC, along with CA1 and dentate gyrus of the hippocampus, and the primary visual cortex, simultaneously with nasal respiratory recordings during spatial navigation in rats. We compared interactions among these areas while rats foraged with either visual or olfactory spatial cues. In both modalities we found high systemwide coherence in two frequency bands matching slow and fast respiratory rates. Granger causality shows dynamic interactions between primary sensory areas depending on the sensory modality of the foraging environment. Phase amplitude coupling analysis shows respiratory and theta coupling at beta and gamma frequencies between olfactory, hippocampal and visual areas. In the second set of experiments, half of the rats from the first experiment were used along with another set of rats in which we also recorded from primary somatosensory, secondary motor, medial parietal association, and medial entorhinal cortices. All rats were trained on a spatial learning task in which they had to navigate to a learned location using either visual or olfactory cues. We compared measures of neural interaction dependent on modality of spatial cues and naïve versus learned states. High levels of coherence were seen between limbic system and sensory cortices at a single frequency around hippocampal theta (~7-8 Hz). Beta (15-35 Hz) and high gamma (60-100 Hz) interactions seen with Granger causality emerged among somatosensory, motor, olfactory, and hippocampal areas during spatial learning which also couple to respiratory and theta rhythms. Our results support the hypothesis that respiration works dynamically with hippocampal theta as part of a master clock for global brain connectivity and help to characterize the role of these coordinated rhythms in cognitive behaviors.

Disclosures: A. Sheriff: None. **L.M. Kay:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.04

Topic: H.09. Spatial Navigation

Support: NIH R01 NS102915
NIH R01 NS097772

Title: Prefrontal-hippocampal interactions are modulated by optogentically paced theta oscillations during the encoding and maintenance phases of spatial working memory

Authors: *S. SRIKANTH[1], Y. HU[1], J. K. LEUTGEB[1], S. LEUTGEB[1,2];
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Abstract: Brain oscillations provide a synchronizing signal for the coordination of spike timing across brain regions, thereby supporting cognitive functions such as working memory. Theta oscillations (7-9 Hz) in the hippocampus, which are generated by pacemaker cells in the medial septal area (MSA), have been found to be important for learning and memory. In addition, interactions between the hippocampus and the prefrontal cortex have been established to be critical for spatial working memory. In this study, we manipulated the precise timing of theta oscillations through optical stimulation of the MSA and investigated the effects of these manipulations on local field potentials and single unit firing patterns in the medial prefrontal cortex. We recorded local field potentials from the hippocampus and medial prefrontal cortex as well as single units from the medial prefrontal cortex while mice performed a delayed spatial alternation task. In mice (n = 6) with channelrhodopsin (ChR2) expression in parvalbumin-positive MSA cells, we show that local field potentials in the hippocampus as well as the medial prefrontal cortex are strongly entrained to optical stimulation of the MSA at accelerated (12 Hz), decelerated (4 Hz) and endogenous (8 Hz) theta frequencies. However, only a subset of local neuronal populations within the medial prefrontal cortex are entrained to the artificially stimulated oscillations (4 Hz: 44% of n=209 cells, 8 Hz: 48% of n=204 cells, 12 Hz: 37% of n=181 cells). Moreover, this entrainment, as measured by the phase locking value, is stronger upon stimulation at 8 Hz compared to 4 Hz or 12 Hz (4 Hz: $p=0.14$, $Z=-1.47$; 8 Hz: $p<.001$, $Z=-3.91$; 12 Hz, $p=0.14$, $Z=-1.45$ Wilcoxon Rank Sum Test). We also found that during spatial working memory the entrainment of mPFC neurons was stronger during the encoding and maintenance phases of a delayed alternation task in comparison to other phases of behavior in response to the 8 Hz and 12 Hz stimulation (Encoding - 4 Hz: $p<.05$, $Z=2.52$, 8 Hz: $p<.001$, $Z=-4.45$, 12 Hz: $p<.05$,$Z=-2.78$; Maintenance - 4 Hz: $p=0.07$, $Z=1.80$, 8 Hz: $p<.001$, $Z=-4.00$, 12 Hz: $p<.05$, $Z=-2.61$; Choice - 4 Hz: $p=0.16$, $Z=1.37$, 8 Hz: $p=0.26$, $Z=-1.12$, 12 Hz: $p=0.33$, $Z=-0.97$, Wilcoxon Rank Sum Test). Spatial firing properties of medial prefrontal cortex neurons remained unaltered in response to stimulation at any of the three frequencies. None of the effects on neural oscillations were observed in control mice with GFP expression in MSA (n = 7) These results suggest that neuronal computations are coordinated by theta oscillations in the medial prefrontal cortex and the hippocampus predominantly during task phases when information is encoded and maintained.

Disclosures: S. Srikanth: None. **Y. Hu:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.05

Topic: H.09. Spatial Navigation

Support: CIHR Grant 155957
NSERC Grant RGPIN-2018-04600

Title: Representational stability in the cortical head-direction circuit

Authors: *S. SKROMNE CARRASCO, G. VIEJO, A. PEYRACHE;
Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Primary sensory cortical areas are characterized by low-level representations of sensory inputs, but whether these representations are stable over a long period of time or whether they are continuously renewed is still unclear. The head-direction (HD) signal is essential for the spatial navigation system. In the cortex, it is processed by the postsubiculum (PoSub). HD cells each fire for a specific direction of the head of the animal in the horizontal plane and constitute a vast majority of PoSub principal neurons. To address the question of representational stability, we used one-photon calcium imaging with portable microscopes ("miniscopes") to longitudinally monitor ensembles of HD cells in the PoSub over periods of weeks in freely moving animals visiting several different environments. In the PoSub, the representation of the HD signal was stable at two levels. First, the pairwise offset between HD neurons was preserved for several weeks, indicating that the subcortical representation of the HD signal is certainly itself stable and that thalamocortical integration is rigid. Second, the orientation of the HD signal at the population level was maintained in a given environment over the same period of time, suggesting that the HD system preserves long-term memories of spatial orientation in different environments. Together, these findings shed light on how spatial information is represented over time in the brain's navigation system.

Disclosures: S. Skromne Carrasco: None. **G. Viejo:** None. **A. Peyrache:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.06

Topic: H.09. Spatial Navigation

Support: CIHR Grant 155957
NSERC Grant RGPIN-2018-04600

Title: Organisation of population activity in the head-direction generator

Authors: *G. VIEJO, S. SKROMNE CARRASCO, A. PEYRACHE;
Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Thalamic head-direction (HD) neurons of the anterodorsal nucleus (ADN) increase their firing rates as a function of the head direction of the animal in the horizontal plane. ADN neurons maintain a coherent signal during all phases of sleep (Peyrache et al, 2015), in agreement with the hypothesis that the system is governed by attractor dynamics. The lateral mammillary nucleus (LMN) is the main input of AD and is believed to be a key structure for HD signal generation. Yet, the internal dynamics of this structure remain unknown. To investigate the organisation and transformation of the population activity in the head-direction generator, we recorded neuronal population simultaneously in LMN and ADN in freely moving mice using silicon probes. Like in ADN, HD neurons in LMN show coherent activity during wakefulness and Rapid Eye Movement (REM) sleep. However, pairwise coordination in LMN was not preserved during non-REM sleep (NREM) in LMN. Specifically, HD neurons in LMN sometimes fire together during NREM sleep even if they fire for opposite directions during wakefulness, as opposed to their downstream reader ADN where coordination is always maintained. These findings show that the HD thalamocortical network exhibits attractor dynamics during NREM sleep, independent of its inputs. Overall, these observations suggest that the thalamus is not a passive relay of incoming signals to the cortex, but plays an active role in sensory processing.

Disclosures: G. Viejo: None. **S. Skromne Carrasco:** None. **A. Peyrache:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.07

Topic: H.09. Spatial Navigation

Support: CIHR Grant 155957

NSERC Grant RGPIN-2018-04600

Title: Dorsoventral gradient of UP-state onset in mouse post-subiculum

Authors: *D. MEHROTRA[1,2], A. J. DUSZKIEWICZ[2,3], D. LEVENSTEIN[2,4], G. VIEJO[2], A. PEYRACHE[2];
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Abstract: Functional gradients along an anatomical axis are a common feature of the spatial navigation system. For example, the medial entorhinal cortex (MEC), containing grid and head-direction (HD) cells, is organized along its dorsoventral (DV) axis. From dorsal to ventral MEC, grid cells show discrete increase in grid spacing and HD cells show gradual decrease in their tuning specificity. These observations beg the question of whether such functional gradients are intrinsic to the MEC or are inherited, at least in part, from their inputs from the post-subiculum (PoSub), which is the main cortical recipient of the thalamic HD signal.
PoSub is a major input to MEC, conveying HD information critical for the generation of the grid code. We focused our investigation on the population activity dynamics during non-Rapid Eye Movement (NREM) sleep, where intrinsic properties of a system can be studied in the absence of sensory inputs.
We used 64-channel linear electrode arrays to conduct simultaneous population recordings along the whole DV axis of the PoSub in adult male mice. NREM sleep is characterized by aperiodic fluctuation of neuronal population activity between activated “UP” states and “DOWN” states during which most cells are hyperpolarized. Preliminary analysis identified reliable sequential activation of PoSub cells at the beginning of UP states as a function of recording depth, where UP states in the dorsal part of PoSub (adjacent to retrosplenial cortex) began earlier than the UP states in the ventral part (adjacent to ventral presubiculum). The spatial distribution of UP state onset could not be solely explained by increased excitability of early firing cells.
UP-DOWN state transitions were near-synchronous along the DV axis, therefore suggesting that the main difference along the axis was the duration of the DOWN states. This could possibly result from a gradient of excitatory recurrence in the network, stronger recurrence leading to shorter DOWN states. In the HD system, excitatory recurrence is expected to play a major role in attractor dynamics and shaping of individual cell tuning. A computational model in which the probability of recurrent excitation was graded along the DV axis recapitulated our main observations.
Overall, these findings demonstrate a gradient of neural dynamics along the DV axis of the PoSub, possibly reflecting a gradient of excitatory collaterals. Further experiments will look at the anatomical and functional relationship between the PoSub and the MEC to provide further insight into the organization of the limbic system and its role in spatial navigation and memory.

Disclosures: D. Mehrotra: None. **A.J. Duszkiewicz:** None. **D. Levenstein:** None. **G. Viejo:** None. **A. Peyrache:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.08

Topic: H.09. Spatial Navigation

Support: CIHR Grant 155957
NSERC Grant RGPIN-2018-04600
Sir Henry Wellcome Postdoctoral Fellowship 206491/Z/17/Z
EMBO Long-Term Fellowship ALTF 382-2017

Title: Functional characterisation of inhibitory head-direction tuning in the cortical head-direction system

Authors: *A. J. DUSZKIEWICZ[1], S. SKROMNE CARRASCO[1], E. OWCZAREK[1], E. BROWN[1], E. R. WOOD[3], A. PEYRACHE[2];
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Abstract: Local inhibition is critical for the maintenance of high-fidelity single-cell tuning across many cortical areas. Furthermore, excitatory-inhibitory recurrent connectivity is thought to support attractor dynamics in many of the brain's spatial navigation systems. However, while the firing patterns of inhibitory interneurons in these areas are known to be spatially non-uniform, not much is known about the principles governing their spatial tuning as well as its contribution to the local attractor dynamics. In order to investigate it, we surveyed the spatial tuning properties of putative fast-spiking interneurons (FS cells) in the cortical head-direction (HD) system - the most fundamental of the brain's allocentric spatial representation systems. To that end, we used 64-channel linear electrode arrays to conduct population recordings parallel to the cortical layers in postsubiculum (PoSub) of freely moving mice (n = 19), and recorded assemblies of up to 174 PoSub cells. We first established that the firing rate of PoSub-FS cells (n = 256) varies by an average of 50% as a function of animal's HD and that this HD tuning is stable both within and across exploration sessions and follows rotation of a distal landmark to the same degree as PoSub-HD cells. What is more, FS cell tuning curves could be recreated from PoSub population activity during wake as well as during sleep, indicating that it is largely tied to the internal dynamics and connectivity within PoSub. We then identified putative synaptically-connected HD-FS cell pairs in the dataset and determined that FS cells receive most of their inputs from HD cells within a 100 μm radius and that FS cell HD tuning reflects the tuning of their presynaptic partners. In order to determine if HD tuning of PoSub-FS cells exhibits any particular patterns, we treated FS cell HD tuning curves as continuous periodic signals and used Fourier analysis to decompose them into component frequencies. We found that only the first three frequency components are over-represented in FS tuning curves in comparison with the

shuffled distribution, illustrating that FS cell tuning curves tend to have one, two or three symmetrically-distributed peaks. Overall, to our knowledge these results constitute the first systematic characterisation of HD tuning in FS cells. We further hypothesise that such patterned tuning of PoSub FS cells serves to enhance the fidelity of the cortical HD signal.

Disclosures: A.J. Duszkiewicz: None. **S. Skromne Carrasco:** None. **E. Owczarek:** None. **E. Brown:** None. **E.R. Wood:** None. **A. Peyrache:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.09

Topic: H.09. Spatial Navigation

Support: NSF NeuroNex 1707287
NIH R01NS121919

Title: Investigation of circuits underlying integration of heading inputs in retrosplenial cortex

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Abstract: Navigation is fundamentally dependent on an accurate internal representation of position and heading. Although position and heading can be maintained in the absence of sensory input, they are continuously updated by sensory information to ensure accuracy. However, it is not well understood how incoming sensory information is incorporated into an internal representation of navigational variables. The mammalian navigation system, particularly the head direction (HD) network, is an ideal framework for investigating this question. The HD network is responsible for generating a heading signal that informs an animal of its orientation relative to its environment. Although a number of sensory inputs contribute to the signal, visual cues are particularly important for anchoring the heading signal to the environment. Here, we use two-photon microscopy to image neurons in the retrosplenial cortex (RSC) of head fixed mice while a physical floating air platform rotates around them to investigate the contribution of visual cues on heading. First, we find robust heading responses in the RSC, even under head fixation, suggesting that non-vestibular sensory information is sufficient to maintain internal heading. Secondly, we sought to understand the contributions of visual cues to the HD network, so we altered the floating air platform to have symmetrical cues, as well as to record in both light and dark periods. We found multiple different response profiles in the RSC, with some cells showing strong coupling to visual cues while others responded to heading independently of cues,

suggesting heterogeneity of cell tuning in the RSC. Finally, we show that one class of these cells integrates visual cues and heading signals to produce a high-fidelity representation of heading in a variety of conditions, suggesting that these cells are a strong candidate for integrating sensory information into the HD network. These results suggest that the heading representation is not entirely dependent on vestibular information over short time periods, and that visual cues contribute to producing a robust and accurate signal in the HD network.

Disclosures: K.K. Sit: None. **M.J. Goard:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.10

Topic: H.09. Spatial Navigation

Support: NIH 1K99NS118112-01
Simons Center for the Social Brain at MIT postdoctoral fellowship
NIH T32GM007753
Center for Brains, Minds and Machines (CBMM) at MIT, funded by NSF STC award CCF-1231216
NIH R01NS106031
NIH R21NS103098
Office of Naval Research

Title: Spatial reasoning via neural dynamics in mouse retrosplenial cortex

Authors: *J. VOIGTS[1], I. KANITSCHEIDER[2], N. J. MILLER[1], E. H. S. TOLOZA[1], J. P. NEWMAN[1], I. R. FIETE[1], M. T. HARNETT[1];
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Abstract: From visual perception to language, sensory stimuli are interpreted differently depending on prior hypotheses. Recurrent dynamics in neural networks have been identified as a mechanism for changing the interpretation of stimuli, but whether the same neural dynamics can form, maintain, and employ hypotheses to interpret ambiguous stimuli is unknown. Here, we show that mouse Retrosplenial cortex (RSC) can integrate information over time to form hypotheses and perform spatial reasoning through recurrent dynamics. We developed a task in which mice navigate using locally ambiguous landmarks that can only be identified through their spatial relationship to each other, requiring sequential refinement of hypotheses. Neurons in RSC and in artificial neural networks (ANNs) trained on the task encoded mixtures of spatial hypotheses, current location, and sensory data, and were constrained by stable attractor

dynamics. The ANN resolved ambiguous landmarks by encoding spatial hypotheses as locations in neural activity space from where identical sensory inputs led to their correct interpretations via stable attractor dynamics; this computation was mirrored in RSC. Our results indicate that interactions between hypotheses and sensory data in recurrent circuits can provide a substrate for complex sequential cognitive processes.

Disclosures: **J. Voigts:** Other; Open Ephys. **I. Kanitscheider:** A. Employment/Salary (full or part-time):; OpenAI. **N.J. Miller:** None. **E.H.S. Toloza:** None. **J.P. Newman:** Other; Open Ephys. **I.R. Fiete:** None. **M.T. Harnett:** None.

Poster

P865. Spatial Navigation and Spatial Memory: Cortical, Hippocampal, and Thalamic Networks

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P865.11

Topic: H.09. Spatial Navigation

Support: NIH R01 6938169
Klingenstein-Simons Fellowship 6938881

Title: Somato-dendritic computations provide a mechanism for landmark-mediated self-localization

Authors: *L. FISCHER, L. XU, K. MURRAY, M. T. HARNETT;
MIT, Cambridge, MA

Abstract: Spatial navigation requires rapid integration of novel sensory information with internal state representations of location. While sensory processing is optimized to rapidly detect behaviorally relevant stimuli, internal state representations must provide stable readouts even if environmental information is scarce. These requirements apply diverging demands on the neural dynamics governing information processing but the mechanisms underlying their interactions remain unknown. Here we tested how sensory inputs are reconciled with self-localization estimates by recording from neurons in retrosplenial cortex (RSC) while mice learned to use visual landmarks to locate rewards in virtual reality. We found a pronounced shift in RSC neurons from representing mostly self-referenced (egocentric) variables to expressing mostly environment-referenced (allocentric) codes that were dependent on behavioral context. A computational model utilizing somato-dendritic interactions in multi-compartment neurons enabled accurate, context-dependent correction of self-localization estimates which were represented by an attractor network. When behavioral context was switched in such a way as to render landmarks irrelevant, activity of both real and model neurons significantly decreased. Single-compartment neurons were unable to switch between behavioral contexts. Our results

propose a new mechanism for context-dependent integration of sensory information with internal state representations in retrosplenial cortex.

Disclosures: L. Fischer: None. **L. Xu:** None. **K. Murray:** None. **M.T. Harnett:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.01

Topic: I.07. Data Analysis and Statistics

Support: NH Grant NINDS U19NS107464

Title: Real-time analysis of neuronal population activity during calcium imaging for informed closed-loop experiments

Authors: *W. LOSERT[1], Z. BOWEN[1], D. DE ZOYSA[3], K. SHILLING-SCRIVO[4], S. AGHAYEE[5], G. DI SALVO[6], A. SMIRNOV[3], P. O. KANOLD[2,7];
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Abstract: The significant post-processing of two-photon imaging data is usually done either after the experiments, or in a rudimentary way during data acquisition. We have developed Neuronal Analysis in Real Time (NeuroART), software that taps into microscope data streams to provide real-time readout of neuronal activity and downstream analysis during image acquisition. NeuroART has been tested on the most widely used two-photon microscopes and operates in parallel with a range of popular microscope software and image formats. The software package allows for the integration of existing image analysis approaches as we demonstrate by integrating the automatic neuronal cell identification routines from CaImAn (Giovannucci et al, ELife 8, e38173 (2019)).
Utilizing NeuroART, experimenters gain knowledge of the data quality in real time and can identify neurons of interest based on quantitative analysis of the ΔF/F traces of each neuron. As a first demonstration of downstream analysis capability, NeuroART employs a pairwise correlation-based analysis to construct functional networks and identify the most population-correlated neurons in the field of view. The downstream analysis within the real-time loop can run in real time on a standard laptop.
We also demonstrate integration of stimulus information on the example of auditory stimuli, which enables real-time quantification of sound encoding in neurons. During the experiment, tones of different frequencies and sound pressure levels are presented repeatedly over many individual trials and the NeuroART application computes the receptive field and tuning of each

neuron within a field of view of the auditory cortex in real time. With these capabilities, NeuroART can enable model-guided experiments for a wide range of microscope platforms.

Disclosures: W. Losert: None. **Z. Bowen:** None. **D. de Zoysa:** None. **K. Shilling-Scrivo:** None. **S. Aghayee:** None. **G. Di Salvo:** None. **A. Smirnov:** None. **P.O. Kanold:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.02

Topic: I.07. Data Analysis and Statistics

Support: PA Department of Health SAP #4100083102
Shanghai Municipal Science and Technology Major Project 2018SHZDZX05
NIH ZIA NS003041

Title: Marmoset Brain Mapping V4: Cortical Parcellation Based on Awake Resting-State fMRI Data

Authors: *X. TIAN[1], Y. CHEN[3], C. TONG[4], P. MAJKA[5], Y. SANZ-PERL[6], D. SZCZUPAK[7], G. DECO[8], M. ROSA[9], Z. LIANG[10], A. C. SILVA[2], C. LIU[11];
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Abstract: The common marmosets (*Callithrix jacchus*), a New World non-human primate, have increased interest in biomedical and neuroscience research and have many advantages for neuroimaging techniques. As a critical open scientific resource for marmoset research, our Marmoset Brain Mapping project (*marmosetbrainmapping.org*) provides a comprehensive set of atlases and analysis tools to facilitate neuroimaging and connectome studies of the marmoset brain. So far, we have released three versions: 1) the cortical parcellation templates based on *ex-vivo* multi-modal MRI (*Liu et al., 2018*); 2) white matter fiber pathways based on ultra-high resolution *ex-vivo* diffusion MRI (*Liu et al., 2020*); and 3) the *in-vivo* population templates and surface analysis tools (*Liu et al., 2021*). Although these versions provide helpful tools to map the neuroanatomy and structural connectome of marmoset brains, a detailed description of their functional neuroanatomy is still lacking. Here, we present the fourth version (v4) of our Marmoset Brain Mapping Project, which provides a functional parcellation of the marmoset

cortex. In addition, in this version, we attempt to solve limitations faced by non-human primate (NHP) data repositories in accelerating the availability of shared, open resources and standards to support neuroimaging studies. The new v4 comprises the following merits: 1) The largest multi-center (with standardized protocols) “awake” resting-state test-retest fMRI dataset, which currently consists of 42 adult marmosets, 62 sessions, and 721 runs (17 min *per* run).2) A novel pipeline for obtaining the cortical functional parcellation, providing a dense connectome, resting-state brain networks, and fine-grained cortical parcellations of marmosets at both the population-level and individual level.3) Test-retest analysis and task-based verification to systematically evaluate the variability of both functional connectivity and cortical parcellations across sites, animals, and sessions.4) Computational modeling tools to examine the relationship between the functional and structural connectivity maps obtained with rs-fMRI, diffusion MRI and neuronal tracing databases. In all, the new Marmoset Brain Mapping v4 and its associated awake rs-MRI data will be an essential open resource for mapping the functional parcellation of the marmoset brain, and to enable large-scale comparative studies of NHP brain architectures.

Disclosures: X. Tian: None. **Z. Liang:** None. **A.C. Silva:** None. **C. Liu:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.03

Topic: I.07. Data Analysis and Statistics

Support: Chan Zuckerberg Initiative Grant

Title: Community sourced AAV Data Hub.

Authors: *J. S. NASSE, A. ABITUA, M. FAN;
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Abstract: The use of AAV vectors in neuroscience research has continued to increase year after year as new tools to view, control, and manipulate the brain are discovered and improved upon. In addition to new functional tools being developed, modifications in AAV capsid proteins and novel promoters have also dramatically increased in recent years. While the expansion of viral vector tools is immensely beneficial to neuroscience research, there are many technical issues that need to be well understood and considered when designing and performing experiments with AAV vectors. Information on the many caveats and nuances in the use of these viral vector tools is often difficult to obtain as negative results and details of troubleshooting efforts are often not published or widely disseminated. In an effort to address this problem, Addgene began beta-testing a community sourced AAV Data Hub (DH) in late 2019. The goal of Addgene’s AAV data hub is to provide a community driven resource for scientists to share experimental details

and caveats about using AAV tools to expand the scientific knowledge base, and to reduce the time and effort spent testing the multitude of variations available for vectors, promoters, and serotypes that a given experiment may require. Despite our initial success in obtaining over 130 data submissions, recent efforts to expand the number of data submissions have been challenging. In an effort to improve and expand the DH we sent a survey to over 5000 researchers that had received AAV vectors from Addgene to determine what the data needs of the community are, assess what motivates scientists to share data, and reveal what inhibits them from sharing data. We received over 180 responses to the survey. An overwhelming number of respondents (~76%) indicated that more data regarding experimental outcomes including negative or unexpected results are desired. The main reason people indicated for not submitting data was a lack of knowledge about the DH (~51%). However, there were also concerns regarding submitting unpublished data (~23%) and concerns over data quality for submission (~28%). Our results highlight a fundamental problem and paradox in the scientific community where there is great need and desire for data that is not commonly published (i.e. negative and troubleshooting data) and a hesitancy to release this type of data. By increasing community awareness and improving incentives for sharing, we seek to build-up this resource to fulfill the needs of the Neuroscience community.

Disclosures: J.S. Nasse: None. **A. Abitua:** None. **M. Fan:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.04

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant U01MH114829
NIH Grant R01NS39600
NIH Grant R01NS086082

Title: Large scale morphology-based similarity search across digital reconstructions of neural arbors

Authors: *B. LJUNGQUIST, M. A. AKRAM, G. A. ASCOLI;
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Abstract: Function of the nervous system depends on neuronal and glial morphology. Continuous advances in imaging, microscopy, and tracing software have provided an increasingly abundant availability of 3D reconstructions of arborizing dendrites, axons, and processes, allowing their detailed study. However, there is a lack of efficient, large-scale

methods to compare and rank their similarity. Using the NeuroMorpho.Org database, we present a similarity search software enabling fast morphological comparison of hundreds of thousands of cellular reconstructions from various species, brain regions, cell types, and preparation protocols. We have compared the performance of different measures of morphology available for these datasets: 1) summary morphometrics calculated by L-Measure, 2) persistence vectors, a vectorized descriptor of branching structure, 3) the combination of the two. For each of these, we also investigated the impact of applying dimensionality reduction using principal component analysis (PCA). We assessed qualitative performance by gauging the ability to rank neurons in order of visual similarity. Moreover, we evaluated information content by examining explained variance and, as a benchmark, the ability to identify occasional duplicate reconstructions of the same specimen. We also investigated the execution time of searches as well as the effect of varying the database size. Our results indicate that the combined method with applied PCA provides an information-rich basis that enables efficient and precise comparison of neural morphology. The performance was also found to be independent of database size and to scale linearly with larger query sizes (number of neurons for which similarity is searched). We have made the similarity search method available as an online software tool both through a user-friendly graphical interface and as an API for machine users.

Disclosures: B. Ljungquist: None. **M.A. Akram:** None. **G.A. Ascoli:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.05

Topic: I.07. Data Analysis and Statistics

Support: NSERC

Title: Inter-method variability in the association between gray matter volume and glutamate levels in the cingulate cortex

Authors: *J. LEE[1], J. ARCHIBALD[2], E. L. MACMILLAN[3], J. L. K. KRAMER[1];
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Abstract: Glutamate, the primary excitatory neurotransmitter in the central nervous system, is of high interest to neuroscientists due to its integral involvement in the transmission and generation of signals. Proton magnetic resonance spectroscopy (^{1}H-MRS), is a non-invasive technique that allows for the measurement of neurochemicals, including glutamate. Previous literature shows glutamate exists in higher concentrations in gray matter (GM) compared to white matter (WM). For this reason, tissue fraction corrections are routine components of MRS analysis pipelines.

Although the positive association between brain GM volume and glutamate concentration is well established, the variability of this relationship resulting from the use of different tissue fraction estimating methods has not been thoroughly investigated. This study examines the relationship between different brain tissue estimating tools and uncorrected levels of glutamate in the cingulate cortex. These tools include neuroimaging softwares FMRIB Software Library (FSL), Advanced Normalization Tools (ANTs), and Statistical Parametric Mapping (SPM). Data were collected from healthy participants (n=5, 2F, age mean=29±6.9, age range=20-39) on a 3T Philips Ingenia Elition with a 32 channel SENSE head coil. Individual FIDs were pre-processed (eddy current correction, frequency alignment, visual inspection) in MATLAB (R2016b). The data was fitted using LCModel (v6.3-1H). To examine the relationship between glutamate levels and tissue fractions, uncorrected glutamate values were used (i.e., institutional units). The 3D T1 data were skull-stripped and segmented into GM, WM, and cerebrospinal fluid (CSF) using each of the neuroimaging softwares. A linear regression analysis on GM volume and glutamate levels in the anterior cingulate cortex (ACC) based on preliminary results revealed the coefficient of determination (R^2) to be 0.06, 0.11, and 0.34 for FSL, ANTs, and SPM respectively. These findings will be further examined in a larger sample size and in three more voxels across the cingulate cortex. Additionally, a deep learning algorithm using a convolutional neural network will be applied, and its performance will be compared alongside the other brain tissue estimating methods (FSL, ANTs, and SPM). The discrepancy in the R^2 values resulting from the use of different neuroimaging softwares exemplifies inter-method variability in the association between GM volume and glutamate levels in the cingulate cortex. This study highlights the importance of cross-validating findings from anatomical images with different neuroimaging tools to ensure accurate and reliable results in future studies.

Disclosures: J. Lee: None. **J. Archibald:** None. **E.L. MacMillan:** A. Employment/Salary (full or part-time):; Philips Health Canada. **J.L.K. Kramer:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.06

Topic: I.07. Data Analysis and Statistics

Support: NIH BRAIN Grant MH114678

Title: Data interface and apps for systems neurophysiology and imaging

Authors: *S. VAN HOOSER, D. GARCÍA MURILLO, O. ROGOVIN, Y. ZHAO, S. CHEN, Z. WANG, Z. KEELEY, D. SHIN, V. M. SUÁREZ CASANOVA, Y. ZHU, L. MARTIN, O. PAPAEMMANOUIL;
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Abstract: New technological advances and a vital industry supporting neuroscience research has resulted in an explosion of techniques and file formats. While these new methods enable innovative and creative experiments, the myriad of data formats and analysis approaches used in different labs makes rapid adoption of these new techniques and sharing with interested third parties, such as theorists, difficult. Standard file formats and standard interfaces are needed to facilitate sharing, reproducibility and rigor in analysis, and distribution of data.
We present a data interface standard for neurophysiological and imaging data. The standard is not a file format but rather is a systematic means of specifying and accessing data used in the neurosciences, including voltage waveforms, imaging data, spike times of neurons, and intensity values in regions-of-interest within imaging data. The data interface standard can be implemented in any programming language. On the one hand, the interface includes tools for reading files from several Multifunction Data Acquisition Devices, 2-photon microscopes, and stimulus devices, and tools for handling the fact that different investigators often organize their data differently on disk. On the other hand, the interface contains a powerful extendible database so that the results of analyses and extracted quantities can be stored and queried. These features allow for an eco-system of best-of-breed "apps" to be developed or converted from existing open source tools, that store standardized results in the database.

Disclosures: S. Van Hooser: None. **D. García Murillo:** None. **O. Rogovin:** None. **Y. Zhao:** None. **S. Chen:** None. **Z. Wang:** None. **Z. Keeley:** None. **D. Shin:** None. **V.M. Suárez Casanova:** None. **Y. Zhu:** None. **L. Martin:** None. **O. Papaemmanouil:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.07

Topic: I.07. Data Analysis and Statistics

Support: NCS-FO: 1835268/1834994
CBMM NSF STC award CCF-1231216

Title: Neurodecoder: an open-source package for reproducible neural population decoding

Authors: *E. MEYERS[1], X. FANG[2];
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Abstract: Neural population decoding is a powerful data analysis method for understanding how the brain processes information (Meyers and Kreiman, 2011). However, running a decoding analysis is significantly more complex than running traditional neural analyses. To make it easier to run decoding analyses, we have previously developed the Neural Decoding Toolbox (www.readout.info) in MATLAB (Meyers, 2013). While the Neural Decoding Toolbox has been

successfully used by a number of research groups (e.g., Schmitt et al. Nature, 2017, Kamiński et al, Neuron 2020, Dotson and Yartsev, Science, 2021), there are additional ways to make running decoding analyses even simpler. In this work we introduce the NeuroDecodeR package, which runs population decoding analyses using the R programming language. Like the Neural Decoding Toolbox, the NeuroDecodeR package is designed in a modular fashion allowing research to try different classifiers and preprocessing algorithms, and to run temporal cross-classification and generalization analyses. The NeuroDecodeR package also makes it easy to develop new methods for quantifying decoding results, to log/save results, and to run code in parallel. Advantages of using the R programming language is that it is free, and there is a rich ecosystem in R for creating reproducible data analysis documents. We have also been developing a web application (a Shiny app) that allows researchers to run reproducible decoding analyses by selection options through a graphical user interface. This platform generates pdfs that contain code and plots of the results (by knitting an automatically generated R Markdown file a pdf), which will further simplify and speed up the time it takes to analyze data.

Disclosures: E. Meyers: None. **X. Fang:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.08

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1840218

Title: Protecting the integrity and provenance of research data with Open Science Chain

Authors: *S. SIVAGNANAM, V. NANDIGAM, M. SHANTHARAM, K. LIN, S. SAKAI; UCSD, La Jolla, CA

Abstract: In neuroscience large amounts of experimental and imaging data are produced in a wide variety of formats and are utilized to develop data-driven computational models for elucidating neuronal and network functions. Investigators whose research involves sophisticated processing of large amounts of these multimodal data require techniques to ensure its integrity, especially when trying to build upon prior research work done by other scientists. When the scientific dataset evolves or is reused in workflows creating derived datasets, the integrity of the dataset, its metadata, and the provenance needs to be securely preserved while providing assurances that they are not accidentally or maliciously altered 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. The metadata entities includes the dataset location (e.g., DOI, URL), description, keywords, and contributor information. 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. Researchers can also link external repositories (e.g., GitHub) and other datasets contributed to OSC to create a detailed workflow of their scientific experiment, linking multiple sources of data and computational code used in their published results.
To lower the complexity barrier for use of this technology and to promote adoption, a web-based portal is developed with seamless user interfaces for interacting with the underlying blockchain. A python-based command line utility is also available that can be used from within a researcher's environment or integrated into research workflows. 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 the confidence of the scientific results.

Disclosures: S. Sivagnanam: None. **V. Nandigam:** None. **M. Shantharam:** None. **K. Lin:** None. **S. Sakai:** None.

Poster

P974. Software Tools III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:15 PM - 4:15 PM

Program #/Poster #: P974.09

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 5R01NS047293-16
Swartz Foundation gift

Title: Actionable event annotation in neuroimaging: HED-3G principles and practice

Authors: *K. ROBBINS;
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Abstract: Introduction: HED-3G is an evolution of the Hierarchical Event Descriptor system to move experiment annotation beyond cryptic keywords and scattered documentation to a standardized annotation of experimental structure that can be directly used by analysis tools to perform sophisticated analyses based on events and other metadata. Rationale: Event markers associate a time-delimited phase of or change in an identifiable process with a defined time point

or period. While event-markers play a central role in organizing neuroimaging and other experimental data, current annotation practice mostly relies on local laboratory strategies for labelling events and other information such as task and experimental design. As a result, data analysts must often resort to assembling information scattered across publications, stored metadata, and internal laboratory documents and must then write custom computer code to perform analyses. Methods: HED-3G provides a vocabulary, software infrastructure, and tools for detailed event annotation. HED-3G standardizes strategies for deciding which events to report, how to annotate them, and how to incorporate the information into analyses and information extraction tools. Researchers using HED-3G first declare the relevant concepts needed to describe their experiment including presented experimental stimuli, participant responses, tasks, and experimental conditions, and describe the temporal structure of their experiment. Researchers then express this information using a standardized, structured vocabulary. The HED-3G infrastructure provides a mapping from defined concepts to event annotations that enables powerful cross-study analysis. Validation tools available online and as Python and JavaScript modules ensure consistency and compliance with the HED-3G specification. The standardized vocabulary and structure allow researchers to use a set of standard tools, eliminating the need for creating customized code. HED-3G is integrated into the BIDS (Brain Imaging Data Structure) system for organizing and sharing datasets. Results: We have developed several case studies that demonstrate the effectiveness of HED-3G in annotating a variety of features and experimental concepts in machine-actionable form. Other aspects of the HED-3G ecosystem are addressed in related posters (Truong: tools), (Makeig: extensions), (Delorme: EEGLAB integration) (Pal Attia: clinical use), (Denissen: fMRI). Conclusions: HED-3G can dramatically improve the utility of neuroimaging and behavioral data. Our challenge is now to encourage researchers to incorporate HED annotation into their new, existing, and shared data.

Disclosures: K. Robbins: None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.01

Topic: A.07. Developmental Disorders

Support: Shriners Hospitals for Children Research Grant 86100 - PHI - 16 to S.K.
Shriners Hospitals for Children Research Grant 85109 - PHI - 18 to S.K.
NINDs Grant R01NS04038 to S.K.

Title: Yap/ Taz dysregulation contributes to brain pathology in tuberous sclerosis complex

Authors: *B. K. TERRY[1,2], R. PARK[1,2], P. B. CRINO[3], S. KIM[1,2];
[1]Shriners Hosp. Pediatric Res. Ctr., [2]Dept. of Neural Sci., Temple Univ., Philadelphia, PA;
[3]Neurol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The genetic disorder Tuberous Sclerosis Complex (TSC) is characterized by the development of benign tumors throughout the body. TSC is caused by mutations in the hamartin (*TSC1*) and/or tuberin (*TSC2*) genes, resulting in hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1) due to reduced Rheb1 inactivation. Brain involvement is seen in the majority of those with TSC and multiple neuropathological features have been previously described. These include the presence of cortical tubers, subependymal nodules, enlarged cells, and disrupted cortical lamination, all of which can contribute to the development of epilepsy and developmental delays. However, while we know that these features are tied to hyperactive mTORC1 signaling, the primary molecular and cellular mechanisms behind the disruptions in brain pathogenesis are not well understood. One pathway that has been recently tied to mTORC1 signaling is the Hippo-Yap/Taz signaling pathway, an evolutionarily conserved pathway that is critical for processes such as organ size control. The purpose of this study was 1) to determine whether Yap/Taz levels are dysregulated in TSC patients and in a TSC animal model and 2) to determine if targeting Yap/Taz could improve TSC-related developmental defects in the cortex of our TSC mouse model. First, to evaluate how mTORC1 hyperactivation impacts Yap/Taz signaling pathway members, we used Western Blotting and immunohistochemistry to measure the levels of these proteins in a human cortical tuber sample, versus control human cortical samples, and in the cortices of *Tsc2* conditional knockout (cKO) animals, versus that of WT controls. Interestingly, we found that total Yap/Taz levels are elevated in both the human cortical tuber sample and in embryonic day 16 *Tsc2* cKO cortexes when compared to control samples. Next, to determine whether reduction of Yap/Taz could rescue the pathological features seen in *Tsc2* cKO animals, we compared the cortical phenotype of *Yap*/*Taz*/*Tsc2* triple conditional knockout (tcKO) animals with *Tsc2* cKO and wild type animals. Of note, we found that *Yap/Taz/Tsc2* conditional knockout had a rescue effect on the cortical thickness increases, cortical/hippocampal lamination defects, and the hypomyelination phenotype associated with *Tsc2* cKO. Taken together, our findings suggest that aberrant activation of Yap/Taz plays an important role in TSC pathogenesis.

Disclosures: B.K. Terry: None. **R. Park:** None. **P.B. Crino:** None. **S. Kim:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.02

Topic: A.07. Developmental Disorders

Support: NIH 1F31HD103339-01
NIH NS046789

Title: Investigation of a synapse-to-chromatin signaling pathway regulating the neuronal BAF complex and dendritic development

Authors: *S. GOURISANKAR[1], W. WENDERSKI[2], S. H. KIM[1], S. G. LIN[1], A. KROKHOTIN[1], J. PAULO[3], J. KWASNICK[1], N. GRAY[1], S. GYGI[3], G. CRABTREE[4]; [2]Dept. of Developmental Biol., [1]Stanford Univ., Stanford, CA; [3]Harvard Univ., Cambridge, MA; [4]Howard Hughes Med. Inst., Stanford, CA

Abstract: Human genetic studies have implicated mutations in the subunits of the neuron-specific chromatin remodeler, nBAF, in autism syndrome disorder (ASD), idiopathic intellectual disabilities (ID), and other neurodevelopmental disorders. In *Drosophila*, similar mutations produce “perfect dendritic retargeting” suggesting a fundamental role for these complexes in synaptic specificity. Sensory experience and synaptic activity shape proper neural development in part by stimulating transcription, which, in turn, is shaped by chromatin remodelers such as nBAF. While previous work has shown that nBAF is necessary for activity-dependent dendritic outgrowth and involved in activity-regulated transcription, we lack clarification of how synaptic activity regulates nBAF. Here, we use proteomic, functional genomic, and biophysical studies in murine cortical neurons to show that synaptic activity leads to rapid, global phosphorylation of the nBAF complex and changes in nBAF remodeling activity. Mutational studies show that phosphorylation of *Smarcc2*, a core nBAF subunit identified as mutated significantly in ASD and ID by large-scale human genetics studies, regulates axon targeting gene expression. Chemical inhibition studies implicate potential nBAF-kinases that link chromatin remodeling with synaptic activity. Our studies help elucidate one mechanism by which synaptic activity regulates experience-driven neurogenesis through chromatin remodeling, and has implications for nBAF-associated neurodevelopmental disorders.

Disclosures: S. Gourisankar: None. **W. Wenderski:** None. **S.H. Kim:** None. **S.G. Lin:** None. **A. Krokhotin:** None. **J. Paulo:** None. **J. Kwasnick:** None. **N. Gray:** None. **S. Gygi:** None. **G. Crabtree:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Foghorn Therapeutics.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.03

Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH109706-02

Title: Characterization of PCM1 isoforms in the adolescent and adult brain

Authors: *C. CHANG[1], L. A. CREW[2], R. YOUNG[1], N. BASAZINEW[1], N. GHANI[1], H. BAKER[1], R. A. WIRT[2], R. GU[1], V. VO[1], R. TILLETT[1], J. M. HYMAN[2], E. OH[1];
[1]Nevada Inst. of Personalized Med., Univ. of Nevada, Las Vegas, Las Vegas, NV; [2]Psychology, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: The pericentriolar material 1 protein (PCM1) is a component of the centriolar satellites and regulates the biogenesis and function of the centrosome and cilium in the nervous system. In response to paracrine signaling, firing patterns in neurons are modified to accommodate changes in the cellular distribution of centriolar satellite proteins at the centrosome. Here, we show that PCM1 spliced isoforms are localized to discrete cellular compartments in neurons and are expressed differentially in the adolescent and adult brain. Using mass spectrometry, we identified protein networks that complex with either the long or short PCM1 spliced isoforms and we show how each isoform can coordinate protein post-translational modifications in tubulin during the development of the brain. Deletion of the PCM1 long isoform results in the modulation of electrical activity, synchrony, and network oscillations in neurons. Electron tomography analyses of synaptic terminals in the adult hippocampus highlight how PCM1 isoform products can modify synaptic vesicle size and density. Finally, electrophysiological recordings from mutant mice demonstrate that PCM1 isoform products are necessary for theta oscillation in the hippocampus. Taken together, our data characterize a new role for centriolar satellite protein isoforms during brain development and identify a potential mechanism for ciliary proteins in human neurological disease states.

Disclosures: C. Chang: None. **L.A. Crew:** None. **R. Young:** None. **N. Basazinew:** None. **N. Ghani:** None. **H. Baker:** None. **R.A. Wirt:** None. **R. Gu:** None. **V. Vo:** None. **R. Tillett:** None. **J.M. Hyman:** None. **E. Oh:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.04

Topic: A.07. Developmental Disorders

Support: Ricerca Corrente 2020/2021 Italian Ministry of Health

Title: Ipsc-derived neural stem cells from joubert syndrome as a novel in vitro model to elucidate ciliopathy-associated molecular mechanisms.

Authors: *J. ROSATI[1], A. D'ANZI[2], G. RUOTOLO[2], A. VESCOVI[2];
[1]Fondazione IRCCS Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy; [2]Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (Foggia), Italy

Abstract: Background: Joubert syndrome (JS) is a rare autosomal recessive condition characterized by a peculiar midbrain-hindbrain malformation, known as the molar tooth sign (MTS). To date, most of the identified causative genes of JS encode proteins involved in cilia function or assembly. We focused our attention on a point mutation of AHI1 (H896R), a gene encoding a protein named Jouberin.Material and Methods. hiPSCs from a healthy donor and a JS patient were produced and fully characterized. In order to study how AHI1 mutation could affect neural differentiation at morphological and molecular level, iPS cells were differentiated in neural cells.Results. Our data show that: 1) The H896R AHI1 mutation leads to a constitutive hyperactivation of Wnt pathway, 2) this mutation causes an incorrect differentiation both in cells morphology and in the balance among neural populations during development.Conclusion. Literature data showed that WNT pathway plays a central role in the estabilishment of dorsal/ventral axis, so we had tested the expression of CNS markers in hiNSC derived from JS patients. The data show that the Joubert's neurospheres have a major expression of dorsal and posterior markers with respect to controls and a constitutive activation of WNT signaling, both canonical and non-canonical. In conclusion, our work confirmes a role for Jouberin in WNT pathway modulation. To date, the functions of primary cilia in human neural stem cells and their changes during neural differentiation remain largely vague, the next goal will be to investigate how the impairment of cilia could influence the neural development.

Disclosures: J. Rosati: None. **A. D'Anzi:** None. **G. Ruotolo:** None. **A. Vescovi:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.05

Topic: A.07. Developmental Disorders

Support: The Slovak Research and Development Agency – APVV-20-0070
Ministry of Education, Science, Research and Sport of the Slovak Republic – VEGA 1/0635/20
Comenius university – UK/33/2021

Title: Expression of intracellular and transmembrane sex hormone receptors during postnatal development of the rat brain

Authors: *M. PILLEROVA, M. PASTOREK, E. RENCZES, P. CELEC, L. TOTHOVA; Inst. of Mol. Biomedicine, Med. Faculty, Comenius University, Bratislava, Slovakia, Bratislava, Slovakia

Abstract: Testosterone (T) affects the structure and the functions of the developing brain. Its metabolism depends on the activity of aromatase and 5α-reductase. T and its metabolites act via intracellular androgen (AR) and estrogen (ERα, ERβ) receptors as well as via novel transmembrane androgen ZIP9 and estrogen GPER1 receptors. The aim of our study was to describe the dynamics of expression of T-related receptors in the brain during early postnatal development. RNA was isolated from the frontal cortex (FC), hippocampus (HIP), hypothalamus (HYP), and cerebellum (CER) of Wistar rats (n=68) of both sexes on postnatal days 1, 7, 14 and 21. Gene expression was analyzed using RT-qPCR with PPIA as the housekeeping gene. T was measured in plasma using ELISA. The concentration of T increased over time from 0,4-0,6 nmol/l to 1,6-2,4 nmol/l. Significant sex differences were found neither in plasma T nor in the gene expression analysis. The expression of the AR increased and peaked on PND=21 (2x-12x in compared to PND=1) in all tested regions. Similarly, ERβ increased and peaked on PND=21 (2x-4x in compared to PND=1) in all tested regions. On the other hand, ERα peaked on PND=1 (3x in compared to PND=21) and decreased in FC and HIP. In the HYP and CER, the expression of ERα increased and peaked on PND=21 (2x-10x in compared to PND=1). In the HIP, the expression of ZIP9 was 2x higher on PND=1 compared to other PNDs. In the FC, HYP, and CER, the dynamics of ZIP9 expression was without a change. Regarding GPER1 expression, in the CER, it increased and peaked on PND21 (6x in compared to PND=1). In the FC, GPER1 was 3x higher on PND=1 in comparison to other PNDs. There was no change in GPER1 dynamics in the HIP and HYP. The 5α-reductase increased over time in CER and HIP, but not HYP and FC, where no dynamics was observed. On the other hand, aromatase decreased in the CER and HYP, increased in the FC, and there was no change in its dynamics in the HIP. In conclusion, the described dynamics of expression of sex hormone receptors and T-metabolizing enzymes may indicate the likely changing signaling pathway induced by T in the developing brain of the rat. Non-classical signaling mediated by transmembrane receptors might be replaced by activation of classical intracellular sex hormone receptors depending on the brain region, but this must be proved in further studies analyzing the expression on the protein level or the activity of the particular pathways. Our findings could be useful for modeling of T-related behavioral disorders.

Disclosures: M. Pillerova: None. **M. Pastorek:** None. **E. Renczes:** None. **P. Celec:** None. **L. Tothova:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.06

Topic: A.07. Developmental Disorders

Support: University of Newcastle RHD Scholarship
NHMRC Grant 1067137
NHMRC Senior Research Fellowship (1121474)

Title: Psychiatric-associated microRNAs are responsive to oxidative stress during neuronal differentiation

Authors: *B. KHAVARI, M. M. BARNETT, E. MAHMOUDI, M. P. GEAGHAN, A. GRAHAM, M. J. CAIRNS;
Univ. of Newcastle, Newcastle, Australia

Abstract: Oxidative stress is suggested as an environmental risk factor for neurodevelopmental and psychiatric disorders, but the underlying mechanisms are not fully understood. We previously showed that the exposure of the neuroblastoma SH-SY5Y cells to oxidative stress before (pre-treatment) or during (co-treatment) differentiation process led to significant changes in the gene expression landscape of the resultant neuron-like cells. The aim of the present study was to examine alterations in microRNA (miRNA) expression as a plausible explanation for those observations, since miRNAs are stress-responsive molecules, able to suppress gene expression at both transcriptional and post-transcriptional levels, and are involved in brain development. Using small RNA-sequencing, we detected differential expression in more than 100 miRNAs, 72 of which were reported before to be dysregulated in psychiatric diseases. Of particular interest was miR-137, which is strongly associated with the genetic risk of schizophrenia, miR-17-5p, dysregulated in several brain regions and peripheral tissues of psychiatric patients, and miR-181b-5p, whose increased expression is one of the most recurrent observations in schizophrenia. Combining the two studies results and calculating the Pearson's correlation coefficient between miRNA-mRNA pairs revealed that differentially expressed miRNAs in both pre- and co-treatment conditions were directly regulating the expression of a subset of genes with implications for critical neurobiological processes, such as neurogenesis, neuron differentiation, and synaptic signalling. In the pre-treatment regimen, miRNA-correlated genes were enriched in neurodevelopmental disorders, whereas in co-treatment condition, cardiovascular and immune diseases, which are highly comorbid with psychiatric disorders, were enriched. Collectively, our findings suggest that early exposure to oxidative stress can considerably affect neuronal differentiation process at the molecular level and possibly increase the risk of psychiatric diseases through perturbing miRNA-gene networks that have critical roles for neurodevelopment.

Disclosures: B. Khavari: A. Employment/Salary (full or part-time):; University of Newcastle. **M.M. Barnett:** None. **E. Mahmoudi:** A. Employment/Salary (full or part-time):; University of Newcastle. **M.P. Geaghan:** A. Employment/Salary (full or part-time):; University of Newcastle. **A. Graham:** A. Employment/Salary (full or part-time):; University of Newcastle. **M.J. Cairns:** A. Employment/Salary (full or part-time):; University of Newcastle.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.07

Topic: A.07. Developmental Disorders

Support: NIH R01 NS102538

Title: Trp53 ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing

Authors: *A. WHITE[1], M. BAUMGARTNER[1,2], M. F. LEE[1], K. D. DRAKE[1], G. S. AQUINO[1], R. KANADIA[1];
[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Genet., Yale Univ., New Haven, CT

Abstract: The minor spliceosome is comprised of 5 small nuclear RNAs (snRNAs) U11, U12, U5, U4atac, and U6atac and is responsible for the splicing of minor introns, which are present in ~700 genes, called minor intron-containing genes (MIGs). Despite being responsible for the splicing of less than 0.5% of introns, mutations in components of the minor spliceosome have been implicated in developmental disorders. For example, minor spliceosome inhibition due to mutations in *RNU4ATAC* is linked to primary microcephaly. We have previously reported that ablation of *Rnu11,* a minor spliceosome specific snRNA, in the developing mouse pallium, inhibits the minor spliceosome resulting in microcephaly. There, cell cycle defects and p53-mediated apoptosis in response to DNA damage resulted in loss of radial glial cells (RGCs), underpinning microcephaly. To test the hypothesis that the cell death, rather than the cell cycle defects drove the observed microcephaly, we ablated *Trp53* to block cell death in the *Rnu11* cKO, to ultimately rescue microcephaly. Here, we report that *Trp53* ablation failed to rescue microcephaly in these double knockout mice (dKO). We show that the transcriptome of the dKO pallium was closer to the control compared to the *Rnu11* cKO. We found aberrant minor intron splicing of MIGs involved in cell cycle regulation, resulting in more severe impaired mitotic progression and cell cycle lengthening of RGCs in the dKO that was detected earlier than the *Rnu11* cKO. We also discovered a potential role of p53 in causing DNA damage in the developing pallium, as detection of γH2aX+ was delayed in the dKO. Thus, we postulate that microcephaly in minor spliceosome-related diseases is primarily caused by cell cycle defects and identify a core set of MIGs that are affected that may be driving the observed microcephaly.

Disclosures: A. White: None. **M. Baumgartner:** None. **M.F. Lee:** None. **K.D. Drake:** None. **G.S. Aquino:** None. **R. Kanadia:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.08

Topic: A.07. Developmental Disorders

Support: 5R01MH102603-04, PA18-586
F31MH123140
HHMI Gilliam Fellowship

Title: Foxp1 regulates the cell cycle in a cell type specific manner in the developing mouse cortex

Authors: *A. ORTIZ, F. AYHAN, M. HARPER, G. KONOPKA;
UT Southwestern, Dallas, TX

Abstract: Cortical development is a tightly controlled process and any deviation may increase the susceptibility to neurodevelopmental disorders, such as autism spectrum disorders (ASD). Numerous studies have identified mutations in FOXP1, a gene encoding a transcription factor enriched in the developing and mature neocortex, as causal for ASD. Our group has previously shown that loss of *Foxp1* in the mouse cortex leads to reduced cortex size, alterations in cortical lamination, and changes in relative thickness of cortical layers. However, the mechanisms underlying these changes remain unclear. My work characterizes the developmental requirement of neocortical *Foxp1* throughout early development using a cortical-specific conditional knock-out of *Foxp1* (*Foxp1* cKO). *Foxp1* expression is temporally regulated in distinct cell types throughout development. In experiments designed to measure cell cycle length in the lateral cortex of *Foxp1* cKO mice, we find that early loss of *Foxp1* results in reduced cell cycle exit of intermediate progenitors but in more rapid cell cycle exit of neuron-fated cells. This provides insight into how Foxp1 regulates cell type generation at an early stage of cortical development. The altered length of cell cycles in specific cell types provides insight into the distinct generation of neurons in *Foxp1* cKO animals to potentially explain the post-natal differences seen in cortical layers and relative cortex thickness. This adds to our ongoing work of genomic profiling, of identifying potential key driver genes, assessing how loss of *Foxp1* alters the proliferative capacity of radial glia cells and cell migration, and cell autonomous and non-autonomous effects.

Disclosures: A. Ortiz: None. **F. Ayhan:** None. **M. Harper:** None. **G. Konopka:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.09

Topic: A.07. Developmental Disorders

Title: Hippocampal gene expression along with autism like behavior and neuroinflammation in offspring male mice from high salt diet fed parents

Authors: *K. AFROZ[1], K. ALVINA[2], N. REYES[3], K. YOUNG[1];
[2]Biol. Sci., [1]Texas Tech. Univ., Lubbock, TX; [3]Texas Tech. Unversity, Lubbock, TX

Abstract: Autism spectrum disorders (ASD) is one of the major neurodevelopmental disorders with characteristic deficit in social behavior as well as impaired learning and executive control. Several recent studies have shown a potential effect of maternal gut microbiota alteration on offspring neurodevelopment in animal models as well as in humans. As the gut microbial population is heavily modulated by dietary habits, offspring ASD has also been associated with maternal diet. Salt is a very common dietary component that has been associated with altered gut microbiota. We previously reported that male offspring from female mice fed with high salt diet (HSD) showed altered postnatal gut microbial composition and altered social behavior and hyperactivity, abnormalities that resemble ASD-like behavior in mouse models. However, the mechanisms behind these behavioral abnormalities are poorly understood.Genomic studies in ASD show networks of genes associated with deregulated synapse development, neuronal activity and altered immune function. Similarly, mouse models such as BTBR, En2-/-, Shank3, show altered gene expression in the hippocampus, including increased microglial genes. Additionally, a recent study showed that postnatal microbiota helps the functional organization of neural circuitry by regulating hippocampal synaptic gene expression and microglial functionality. Our previous data showed that offspring from HSD-fed female mice show significantly decreased level of *Lactobacillus spp*., change that has been associated with gene expression alterations in mouse brain and social behavior deficit as well as neuroinflammation. Therefore, we hypothesized that the altered postnatal microbiota in the offspring of HSD-fed mother alters expression of major hippocampal gene pathways that are associated with ASD-like behavior and neuroinflammation. To test the hypothesis, we are currently doing total transcriptome analysis of the hippocampus collected from the offspring of HSD and control fed mothers. Further, next generation RNA-sequencing is being used to obtain the differentially expressed genes (DEGs) . The ingenuity pathway analysis (IPA) software is being used to identify biological pathways, processes associated with the differentially expressed genes. In addition, to identify neuroinflammation markers in the hippocampus, we used immunohistochemistry in fixed brain samples from offspring from HSD-fed mothers. Our results will contribute to understand how environmental factors such as diet can influence brain development.
1

Disclosures: K. Afroz: None. **K. Alvina:** A. Employment/Salary (full or part-time):; Department of Neuroscience, University of Florida, Gainesville, FL 32610. **N. Reyes:** None. **K. Young:** None.

Poster

P030. Neural Development: Molecular Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P030.10

Topic: A.07. Developmental Disorders

Title: Fibcd1 is a conserved receptor for CS-4S of the brain's extracellular matrix

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Abstract: *Fibrinogen C Domain Containing 1* (*FIBCD1*) encodes a single-pass, type II transmembrane lectin protein with an endocytic function, with no link to nervous system function yet published. Previous work has shown *FIBCD1* to recognise the chitin component of fungal cell walls and a role in the innate immune response to fungal infection. However, publicly available RNA-seq datasets and our own data show *FIBCD1* to be expressed throughout life in neurons of the human and mouse brain, particularly the hippocampus, raising the question: does *FIBCD1* have a role in neurodevelopment? By utilising knockout (KO) mouse and knockdown (KD) *D.melanogaster* models, we show that FIBCD1 functions at the interface between neurons and the brain's extracellular matrix (ECM), specifically chondroitin sulphate proteoglycans (CSPG) with sulphation at carbon 4 (CS-4S). We found that *FIBCD1* is a mediator of CSPG-neuron signalling and that the absence of *FIBCD1* leads to accumulated CS-4S and disruption to hippocampal synaptic plasticity. Finally, through whole-exome sequencing, we have identified loss of function homozygous or compound heterozygous variants in *FIBCD1* in 2 unrelated patients with severe neurodevelopmental disorder. *In vitro,* CSPG coatings inhibited attachment of *Fibcd1*-WT primary hippocampal neurons as well as inducing soma aggregation and dendrite fasciculation (in line with previous work). This was not the case for *Fibcd1*-KO neurons, indicating that *Fibcd1* is required for neuronal responses to CSPG signalling. We found that KO mice exhibited deficiencies in learning tasks, such as inhibitory avoidance, and electrophysiological recordings of hippocampal slices revealed a concordant disruption of LTP

and paired-pulse facilitation. In addition, we found an aberrant accumulation of CS-4S in the hippocampus of the KO mouse, likely due to the lack of Fibcd1's endocytic activity. We pre-treated the hippocampal slices with the enzyme Chondroitinase ABC (ChABC), to digest CSPGs, which fully rescued the deficient LTP and paired-pulse facilitation in the KO slices. Finally, we investigated if *Fibcd1*'s role is conserved among other organisms by generating *Drosophila* lines with RNAi-mediated KD of a putative Fibcd1 orthologue, *CG10359*. While full body KD was lethal, KD of *CG10359* in neurons during development resulted in perturbed neuronal morphology at the neuro-muscular junction as well as deficiencies in climbing assay performance. In summary, our study identifies *FIBCD1* as key to neurodevelopment. We show *FIBCD1* to function at the interface of hippocampal neurons and the ECM and identify *FIBCD1* as a novel candidate gene causative of neurodevelopmental disorder.

Disclosures: C.W. fell: None. **A. Hagelkruys:** None. **A. Cicvaric:** None. **M. Horrer:** None. **L. Liu:** None. **J.S.S. Li:** None. **J. Stadlmann:** None. **A. Polyansky:** None. **S. Mereiter:** None. **M.A. Tejada:** None. **T. Kokotovic:** None. **A. Scaramuzza:** None. **K. Twyman:** None. **M.M. Morrow:** None. **J. Juusola:** None. **H. Yan:** None. **J. Wang:** None. **M. Burmeister:** None. **T.L. Andersen:** None. **G. wirnsberger:** None. **U. holmskov:** None. **N. perrimon:** None. **B. zagrovic:** None. **F.J. Monje:** None. **J.B. Moeller:** None. **J.M. penninger:** None. **V. nagy:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.01

Topic: A.09. Adolescent Development

Support: K99 AT010903-01A1

Title: Altered environmental lighting during adolescence induces neurotransmitter switching in the medial amygdala affecting anxiety-like behaviors in mice

Authors: *A. PORCU, A. NILSSON, S. BOOREDDY, D. DULCIS;
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Abstract: Background: Anxiety and mood disorders are the most common emotional disorders in adolescents. Adolescence is a remarkable period of neural plasticity in the amygdala, a well conserved structure best known for its role in emotional behaviors. Although in many cases adaptive, the capacity for neural circuit alteration also induces a state of vulnerability to environmental perturbations. In modern societies, adolescents experience increased artificial light exposure, as 80% of adolescents in the USA reported using smartphones and tablets at night. Altered environmental light is associated with impaired mood and an increased risk of

psychiatric disorders. The medial amygdala (MeA), a sub-nucleus of the amygdala, is situated in a crucial position to link light information to brain regions regulating anxiety and risky behaviors. However, the cellular and molecular mechanisms behind the effects of light on brain function is still incomplete. We hypothesized that exposure to altered environmental lighting during adolescence affects emotional responses through a mechanism of MeA neuroplasticity.Methods: We developed a new light paradigm to mimic human adolescent light exposure in mice. This model consists of a prolonged light phase (19hrs/day), with light appearing during the biological night phase of the mice. Adolescent mice were exposed to either such light protocol or to control conditions (12hrs/day) for 4 weeks, then tested for anxiety-like behavior. Immunohistochemical analysis was performed to reveal any changes in neurotransmitter phenotype in the MeA. Chemogenetic (DREADDs)-mediated approach was used to establish a causal relationship between light-induced neurotransmitter switching in the MeA and anxiety-like behavior.Results: Exposure to altered environmental lighting increased anxiety-like responses and risky behavior in adolescent mice. The number of GABAergic and somatostatin neurons in the MeA was significantly different in adolescent mice exposed to the altered environmental lighting compared to controls. Chemogenetic inhibition of MeA GABAergic neurons interfered with light-mediated neurotransmitter switching in the MeA, and reduced anxiety states in adolescent mice.Conclusions: Our data indicate that altered environmental lighting induces changes in neurotransmitter expression in MeA neuronal populations, and that such neuroplasticity contributes to the vulnerability to anxiety and risky behaviors in adolescent mice. Overall, our study reveals potential molecular targets for antianxiety interventions and new strategies to advance mental health in human adolescents.

Disclosures: A. Porcu: None. **A. Nilsson:** None. **S. Booreddy:** None. **D. Dulcis:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.02

Topic: A.09. Adolescent Development

Support: Start-Up Funds from Bowling Green State University to JW

Title: A role for pubertal onset in changes in midbrain dopamine neuron number during adolescence

Authors: *M. G. WOODBURN, V. R. RIESGO, K. A. S. FLANIGAN, J. WILLING; Psychology, Bowling Green State Univ., Bowling Green, OH

Abstract: The ventral tegmental area (VTA) and substantia nigra (SN) of the midbrain produce the majority of dopamine in the brain, making these regions important for mood, sexual

behavior, cognition, controlled movements, and other behaviors. Cell number in the midbrain fluctuates throughout the lifespan, and recent research suggests notable changes during adolescence in terms of the number of dopamine neurons and the total number of neurons. While previous work has documented the effects of pubertal hormones, such as estrogen and testosterone, on dopaminergic functioning and cell number, there have been no studies examining changes in dopamine neurons in relation to the timing of puberty. In the present study, we collected midbrain tissue from pre-pubertal and post-pubertal adolescent rats. We aimed to assess whether cellular changes in the midbrain occur linearly across age, or whether pubertal onset may be a factor. In both males and females, we used stereological methods to document changes in dopamine neuron number, total neuron number, and the percentage of dopaminergic neurons in the VTA and SN. Our results suggest that puberty may be an important factor in the cellular maturation of the dopaminergic midbrain, which may shed light on the observed sex differences in psychiatric illnesses that tend to manifest during adolescence.

Disclosures: M.G. Woodburn: None. **V.R. Riesgo:** None. **K.A.S. Flanigan:** None. **J. Willing:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.03

Topic: A.09. Adolescent Development

Support: NIH grant P60AA011605
T32 grant AA007573

Title: Adolescent alcohol exposure increases perineuronal net density but not parvalbumin or cholinergic interneuron counts in the prefrontal cortex of adult rats.

Authors: *C. DANNENHOFFER[1], G. A. GOMEZ ACOSTA[2], V. A. MACHT[2], R. JAWAD[1], E. SUTHERLAND[1], R. P. VETRENO[3], F. T. CREWS[5], C. A. BOETTIGER[6], **D. L. ROBINSON**[4]; [1]UNC Chapel Hill, Chapel Hill, NC; [2]Bowles Ctr. for Alcohol Studies, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [3]Sch. of Med., [4]Bowles Ctr. for Alcohol Studies, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [5]Prof Pharmacol & Psychiat, Skipper Bowles Ctr. Alcohol, Chapel Hill, NC; [6]Psychology & Neurosci., Univ. of North Carolina, Chapel Hill, NC

Abstract: Binge alcohol exposure during adolescence results in long-lasting alterations in brain and behavior. Specifically, rodent studies show that adult animals with a history of adolescent intermittent ethanol (AIE) exposure exhibit deficits in cognitive flexibility, along with a variety of neurochemical, molecular and epigenetic alterations in brain regions underlying behavioral

choice. One well-described effect of AIE is reduced choline acetyltransferase (ChAT) expression in the basal forebrain, reflecting impaired cholinergic phenotype. As acetylcholine is critical for cognition, AIE-induced reductions in ChAT may contribute to observed cognitive deficits, but it is unclear whether cholinergic interneurons in the prefrontal cortex (PFC) are affected similarly to cholinergic neurons in the basal forebrain that project to the PFC. We also found that AIE reduced functional connectivity via resting-state MRI among PFC and striatal regions. While parvalbumin (PV)-positive interneurons are known to contribute to neural synchrony, it is unclear whether AIE impacts PV neurons. Thus, the present study addressed the hypothesis that AIE-induced behavioral and connectivity deficits are due in part to persistent alterations in expression of PV and ChAT interneurons in key prefrontal and striatal brain regions. We used immunohistochemistry to measure expression of ChAT, PV and perineuronal nets (PNN) that regulate PV neuronal plasticity. Male rats were exposed to AIE (5 g/kg/day, 2-days-on/2-days-off, IG, P25-P54) or water (CON) and brain tissue was harvested in adulthood (P80). While AIE reduced ChAT-positive cell counts in the basal forebrain, they were unchanged in the striatum or PFC. This finding indicates that cholinergic projections to the PFC likely affect AIE-induced behavioral changes rather than cholinergic interneurons. PV cell counts were also similar between AIE-exposed and CON rats in the striatum and PFC. However, we found that WFA expression, a marker of PNN, was increased in the PFC of AIE-exposed rats compared to CON (WFA expression was minimal in striatum). Finally, we used immunofluorescence to assess co-expression of PV and WFA and found that significantly more PV neurons were surrounded by WFA in AIE-exposed (approx. 40 percent) versus water-exposed rats (approx. 35 percent). These findings indicate that while PV neuron numbers are not altered by AIE exposure, their function may be altered via extracellular regulatory structures such as PNN. AIE-induced increases in PNN density surrounding PV interneurons may restrict plasticity in PFC microcircuits and contribute to behavioral deficits reliant upon frontostriatal connectivity.

Disclosures: C. Dannenhoffer: None. **G.A. Gomez Acosta:** None. **V.A. Macht:** None. **R. Jawad:** None. **E. Sutherland:** None. **R.P. Vetreno:** None. **F.T. Crews:** None. **C.A. Boettiger:** None. **D.L. Robinson:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.04

Topic: A.09. Adolescent Development

Support: 1R21MH125367

Title: Late maturing neurons in juvenile mice share features of immature neurons in the human amygdala

Authors: *P. ALDERMAN[1], C. E. PAGE[1], L. TORRIJOS[2], D. SAXON[3], S. VICINI[3], J. CORBIN[4], J. GARCÍA VERDUGO[2], V. HERRANZ PÉREZ[2], S. F. SORRELLS[1];
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Abstract: In the human amygdala, a center for emotional salience processing, the paralaminar nucleus contains a large population of immature excitatory neurons that undergo a delayed maturation after birth, occurring primarily during childhood and adolescence. Similar pools of immature neurons are present in multiple brain regions in many mammals; however it is not clear if any immature neurons in mice are homologous to immature amygdala neurons in humans. A mouse model of these cells would permit experimentation to investigate the development and function of this form of neurodevelopment. We examined brain regions containing immature doublecortin-expressing neurons in mice between birth and adulthood using light and electron microscopy to identify their molecular and morphological features. We identified a subset of immature neurons that express many of the same transcription factors as the immature neurons in the human paralaminar nuclei,and have similar morphology and ultrastructural features. Using unbiased stereology and the optical fractionator across postnatal ages, we find that these neurons decline in number progressively over postnatal life. Thymidine analog birthdating labels these cells when given to timed pregnant females during embryonic stages, suggesting that they are born embryonically, despite their persistent immature state. These late maturing neurons grow during critical times when cognitive functions mature, and therefore may have an important role in circuit plasticity. This work provides a foundation for better connecting investigations of mouse late maturing neurons to similar cells in humans.

Disclosures: P. Alderman: None. **C.E. Page:** None. **L. Torrijos:** None. **D. Saxon:** None. **S. Vicini:** None. **J. Corbin:** None. **J. García Verdugo:** None. **V. Herranz Pérez:** None. **S.F. Sorrells:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.05

Topic: A.09. Adolescent Development

Support: NIH Grant R01DA049737
NIH Grant R01DA02912

Title: E-cigarette exposure during pregnancy disrupts postnatal blood-brain barrier integrity and deteriorates aspects of learning, memory and motor function

Authors: *S. RAHMAN ARCHIE, A. SIFAT, H. VILLALBA, S. SHARMA, S. NOZOHOURI, T. ABBRUSCATO;
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Abstract: Even though electronic cigarettes (e-Cig) has been perceived as a safer alternative for pregnant women, there is a growing concern related to its impact on neonates. Several studies have shown the harmful effects of prenatal tobacco smoking on postnatal health however, no such data is available in case of e-Cig exposure during pregnancy. Due to the noticeable growth in e-Cig usage during pregnancy, it has become crucial to study the impact of maternal e-cig smoking on postnatal health outcomes. Hence, in this study, we have evaluated the consequences of prenatal e-Cig use during pregnancy on postnatal development of blood-brain barrier (BBB) and on behavioral outcomes of adolescent and adult offspring. Pregnant CD1 mice (E5) were exposed to e-Cig vapor (2.4% nicotine) till postnatal day (PD) 7. Weight of the offspring was measured at different time-points till PD 90. Nicotine and cotinine concentration in plasma and brain were measured in PD 7 offspring by LC-MS/MS. The expression level of structural elements of BBB, tight junction proteins (ZO-1, Claudin-5, Occludin), astrocyte (GFAP), pericyte (PDGFRβ) and basement membrane (Laminin α1, Laminin α4) were analyzed in offspring using western blot and immunohistochemistry at PD 7, PD 23, PD 45 and PD 90. Long-term motor and cognitive functions were evaluated using open field test, novel object recognition test and morris water maze test at adolescence and adult age. Reduced body weight was observed in e-Cig exposed offspring at all time points till PD 90 compared to control ($P < 0.05$). Plasma concentration of nicotine and cotinine in PD 7 offspring were 3.215 ng/mL and 3.373 ng/mL respectively whereas in brain, these two concentrations were 43.72 ng/mL and 1.123 ng/mL respectively. Significantly reduced expression of tight junction proteins was found in male and female offspring at PD 7, PD 23, PD 45 and PD 90 ($P < 0.05$). Additionally, prenatally e-Cig exposed both male and female adolescent and adult offspring showed impaired locomotor, spatial learning, and memory function compared to control offspring ($P < 0.05$). Our findings indicate that prenatal e-Cig exposure disrupts postnatal blood-brain barrier integrity by downregulating tight junction proteins' expression and impairs motor and cognitive function at adolescence and adult age.

Disclosures: S. Rahman Archie: None. **A. Sifat:** None. **H. Villalba:** None. **S. Sharma:** None. **S. Nozohouri:** None. **T. Abbruscato:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.06

Topic: A.09. Adolescent Development

Title: Developmental influences on the initial subjective rewarding effects of ethanol in mice

Authors: *M. A. WALDRON[1], H. JONES[1], M. SCOPANO[1], E. M. RHINEHART[2], J. E. GRISEL[1];
[1]Bucknell Univ., Lewisburg, PA; [2]Susquehanna Univ., Selinsgrove, PA

Abstract: There is a growing recognition that adolescent exposure to addictive drugs significantly increases the risk of drug dependence later in life, in part due to the neuroadaptive changes that underlie alcohol use disorders (AUD) (Nixon & McClain, 2010). Additionally, variations in b-endorphin (βE) levels contribute to the subjective sensitivity to the rewarding effects of alcohol. Low basal levels of βE are correlated with increased risk for AUDs (Dai et al., 2005; Kiefer et al., 2006), and studies involving reduced endorphin levels demonstrate the tendency for increased ethanol intake in both rodents and humans (Grahame et al., 2000; Grisel et al., 1999; McGonigle et al., 2016). The present studies were aimed to better understand developmental contributions to the risk for disordered drinking, and especially as they might depend upon sex and βE, in order to facilitate the long-term goal of developing effective interventions for individuals at high risk for AUDs. Experiment 1 assessed the effect of adolescent pre-exposure to ethanol on adult single-exposure conditioned place preference (SE-CPP; Grisel et al., 2014), in male and female, control and βE deficient mice. Between PND 28-35, Adolescent C57BL/6J (WT) and βE deficient (KO) mice were injected once, intraperitoneally, with 1.5g/kg of ethanol or saline. Between PND 70-90, we employed a SE-CPP to investigate the impact of EtOH pre-exposure on the initial subjective rewarding effects of the drug in mice. Pre-exposure to EtOH during the adolescent period reduced sensitivity to the rewarding effects of ethanol in KO mice and increased sensitivity to alcohol-induced sedation in these mice. Pre-exposure did not affect EtOH reward or sedation in WT control mice. Experiment 2a assessed age-related differences in the initial subjective rewarding effects of 1.5 g/kg ethanol and the role of sex and βE. We tested naive adolescent (4-5 weeks) and adult (10-12 weeks) male and female, WT and KO mice. CPP was dependent on both age and βE, in that KO mice of either age, as well as adult WT, were more sensitive to the rewarding effects of ethanol; adolescent WT appeared to be insensitive to the initial rewarding effects of EtOH. Additionally, all adolescent mice failed to show alcohol-induced sedation. Experiment 2b examined the neurochemical differences in WT and KO, adolescent and adult mice from Experiment 2a. Although there were no signs of sex differences in the previous experiments, results indicated that neuronal activation to the CPP paradigm in brain regions associated with addiction was dependent on both age and sex. Taken together these results suggest that low endorphin and age differentially impact the vulnerability to AUDs.

Disclosures: M.A. Waldron: None. **H. Jones:** None. **M. Scopano:** None. **E.M. Rhinehart:** None. **J.E. Grisel:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.07

Topic: A.09. Adolescent Development

Title: Daily Treatment with a GnRH During the Peri-Adolescent Period Delayed Puberty but Failed to Disrupt Estrous Cyclicity or Sexual Motivation in Female Rats

Authors: *F. A. GUARRACI[1], L. AVENDANO[2], M. KELLY[2], B. SENCHEREY[1], N. DHINAKER[1], L. K. DAVIS[1], H. VALDIVIA[1], C. ESTOESTA[1], B. FROHOCK[1], A. C. GORE[3]; [1]Psychology, [2]Southwestern Univ., Georgetown, TX; [3]Col. of Pharm., Univ. of Texas at Austin, Austin, TX

Abstract: The present study examined the long-term effects of suppressing puberty with a GnRH agonist on reproductive physiology and behavior in female rats. Little is known about the long-term effects of chronic GnRH agonist treatment on reproductive physiology and behavior when treatment starts prior to puberty and continues until early adulthood in animal subjects. We have recently reported that administration of the GnRH agonist leuprolide acetate (25 µg/kg) daily for 25 days (postnatal day (PD) 25-50) delayed puberty and disrupted the development of copulatory behavior and sexual motivation in male rats. However, pilot data from our lab suggest that this dose of leuprolide acetate was not high enough to significantly delay puberty in female rats. Therefore, we injected female Long-Evans rats with either leuprolide acetate at a higher dose (50 µg/kg) or 0.9% sterile saline daily for 25 days, starting on PD 25 and ending on PD 50. Vaginal opening was observed daily starting on PD 30 for signs of pubertal onset and first estrous cycle. In addition, we measured estrous cyclicity in these adult females, approximately 2 weeks after last injection of leuprolide (8-10 weeks old). Starting immediately after monitoring estrous cyclicity, females were mated on their first day in behavioral estrous using the partner preference paradigm, with and without physical contact. We found that this higher dose of leuprolide significantly delayed puberty in female rats. However, neither estrous cyclicity nor sexual motivation was significantly affected by a history of leuprolide administration. When considered with our previous findings in male rats, these results add to understanding of the developmental effects of chemically suppressing puberty.

Disclosures: F.A. Guarraci: None. **L. Avendano:** None. **M. Kelly:** None. **B. Sencherey:** None. **N. Dhinaker:** None. **L.K. Davis:** None. **H. Valdivia:** None. **C. Estoesta:** None. **B. Frohock:** None. **A.C. Gore:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.08

Topic: A.09. Adolescent Development

Support: NIH-R01DA037911
Canadian Cannabis and Psychosis research team grant. CIHR MOP-431097

Title: Tetrahydrocannabinol in adolescence dysregulates signaling pathway that orchestrates dopamine development

Authors: *G. HERNANDEZ[1], **T. CAPOLICCHIO**[1], M. GIROUX[2], K. ESTRADA[3], C. FLORES[4];
[1]Douglas Mental Hlth. Univ. Institute/Mcgill, Verdun, QC, Canada; [2]Douglas Res. Inst., Montreal, QC, Canada; [3]McGill Univ., Montreal, QC, Canada; [4]Dept of Psych, McGill Univ., Verdun, QC, Canada

Abstract: Cannabis is one of the most consumed substances by adolescents in North America and its regular use during this developmental period is linked to increased risk of cognitive impairment and psychopathology later in life. Yet, there is a gap in knowledge regarding the cellular and molecular processes underlying adolescent brain development and how they are influenced by exposure to drugs of abuse. Dopamine axons continue to grow from the nucleus accumbens to the prefrontal cortex throughout adolescence, remaining susceptible to environmental changes. Protracted mesocorticolimbic dopamine maturation is mediated by the Netrin-1 guidance cue receptor, DCC (deleted in colorectal cancer), whose expression is controlled by the microRNA, miR-218. Our studies in male mice have shown that stimulant drugs of abuse in adolescence dysregulate miR-218 in the ventral tegmental area (VTA), altering *Dcc* mRNA levels and inducing changes in mesocorticolimbic dopamine, consequentially impacting cognitive control in adulthood. Here we assessed whether intraperitoneal injections of tetrahydrocannabinol (THC) (0, 2.5, 5, 10 mg/kg), every other day, in early adolescence (PND 22- PND30) alters miR-218 and *Dcc* mRNA expression in the VTA in male and female mice. Exposure to the 5 and 10, but not the 2.5 mg/kg dose, downregulates miR-218, inducing a concomitant increase in *Dcc* mRNA levels. These changes seem to be absent in adolescent female mice. Furthermore, THC exposure leads to a dose-dependent reduction in body weight in males; this effect is only observed in females treated with the highest dose. Using the Go/No-Go task, we are currently examining whether adolescent exposure to THC impacts behavioral inhibition in adulthood. These results indicate that early adolescence is a vulnerable period during which exposure to THC produces changes in the molecular signaling controlling mesocorticolimbic dopamine maturation. In previous research these changes have been associated with alterations in behavioral inhibition and cognitive control. Altogether these factors may contribute to enhanced risk to addiction.

Disclosures: G. Hernandez: None. **T. Capolicchio:** None. **M. Giroux:** None. **K. Estrada:** None. **C. Flores:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.09

Topic: A.09. Adolescent Development

Support: NSERC Discovery Grant 203596-13

Title: Sex differences in prepubescent rats following a heterotypic stress paradigm: Are CB1 receptors contributing to post stress sociability and fear responses?

Authors: *E. PERSON[1], A. MORIN[1], V. BOISVERT[2], H. PLAMONDON[3];
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Abstract: Early adolescent stress exposure has been associated with reduced social interaction, especially in male rats, and effects on cognitive functions in both sexes. Notably, studies have suggested a role of the endocannabinoid system in memory and stress, with studies mostly focusing on male's responses. However, research support females to respond differently to stress. This study therefore aimed to investigate the sex differences in social behaviours and memory in prepubescent rats following a heterotypic stress paradigm and an acute dose of a CB1 antagonist (AM251). Female and male Wistar rats (N = 64; aged postnatal day [PND] 30) were randomly assigned to either the stress or no stress condition at the beginning of the heterotypic stress paradigm. The heterotypic stress consisted of a 10-day paradigm alternating between 30 min of restraint stress on even numbered days, and 15 min of forced swim on odd numbered days. Animals in the stress condition were stressed in the morning, while animals in the no stress condition were handled gently. Starting three days after the end of the heterotypic stress, animals were tested in the social interaction and preference tests (SIT and SP) on PND42, and in the Y-Maze passive avoidance test (YMPAT) on PND44. One hour before each test, animals in each experimental groups were injected with AM251 (1mg/kg; i.p) or received saline. Our findings indicated no significant sex differences in social recognition, although a trend was observed for the stress and the AM-251 conditions to interact in prepubescent rats. In the YMPAT, females generally took more time to exit the starting arm than males, specifically in the first trial, which could be an indication of increased anxiety in female towards novelty.

Disclosures: E. Person: None. **A. Morin:** None. **V. Boisvert:** None. **H. Plamondon:** None.

Poster

P074. Animal Models IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P074.10

Topic: A.09. Adolescent Development

Support: NIH R01MH086507
R01MH105488
CIHR 201811MFE-415573-64156

Title: Maturation of basolateral amygdala-prefrontal functional connectivity and conditioned fear during adolescence

Authors: M. AUGER, K. TSENG;
Anat. & Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Basolateral amygdala (BLA) inputs to the prefrontal cortex (PFC) are thought to convey emotional information necessary for responding adaptively to threat. Anatomical studies have suggested that the density of BLA afferents in the PFC increases during adolescence, which may consequently underlie changes in fear behavior during the transition from adolescence to adulthood. Here, we investigated whether a functional refinement in BLA-PFC connectivity occurs using local field potential (LFP) recordings that assess PFC responses to BLA input in rats from postnatal day (P) 30 to adulthood. We also examined whether maturation of BLA-PFC functional connectivity is accompanied by a corresponding change in behavior during the adolescent transition to adulthood in the auditory delay fear conditioning paradigm, which is thought to require BLA input to PFC. Data revealed that the characteristic LFP facilitation observed in the adult PFC following 15 Hz BLA stimulation was not fully expressed until after P60 in naïve rats. Concurrently, the level of LFP suppression at 40 Hz does not reach maximal amplitude until P60, indicating that local PFC excitatory and inhibitory responses to BLA inputs mature through adolescence with distinct developmental trajectories. PFC LFP facilitation to 15 Hz BLA stimulation was dependent on both GluN2A and GluN2B NMDA receptor signaling, while suppression of PFC LFP responses to 40 Hz BLA stimulation requires intact PFC GABAergic transmission, and PFC GluN2A, but not GluN2B, NMDA receptors in adult rats. We observed reduced freezing in adolescent rats in comparison to adults during acquisition of the delay fear task. Reduced freezing in adolescents relative to adults was also observed during cue extinction and during a test of contextual fear memory. Collectively, our findings identify a novel feature of PFC functional refinement that may support the development of mature responding to threat during the progression from adolescence to adulthood.

Disclosures: M. Auger: None. **K. Tseng:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.01

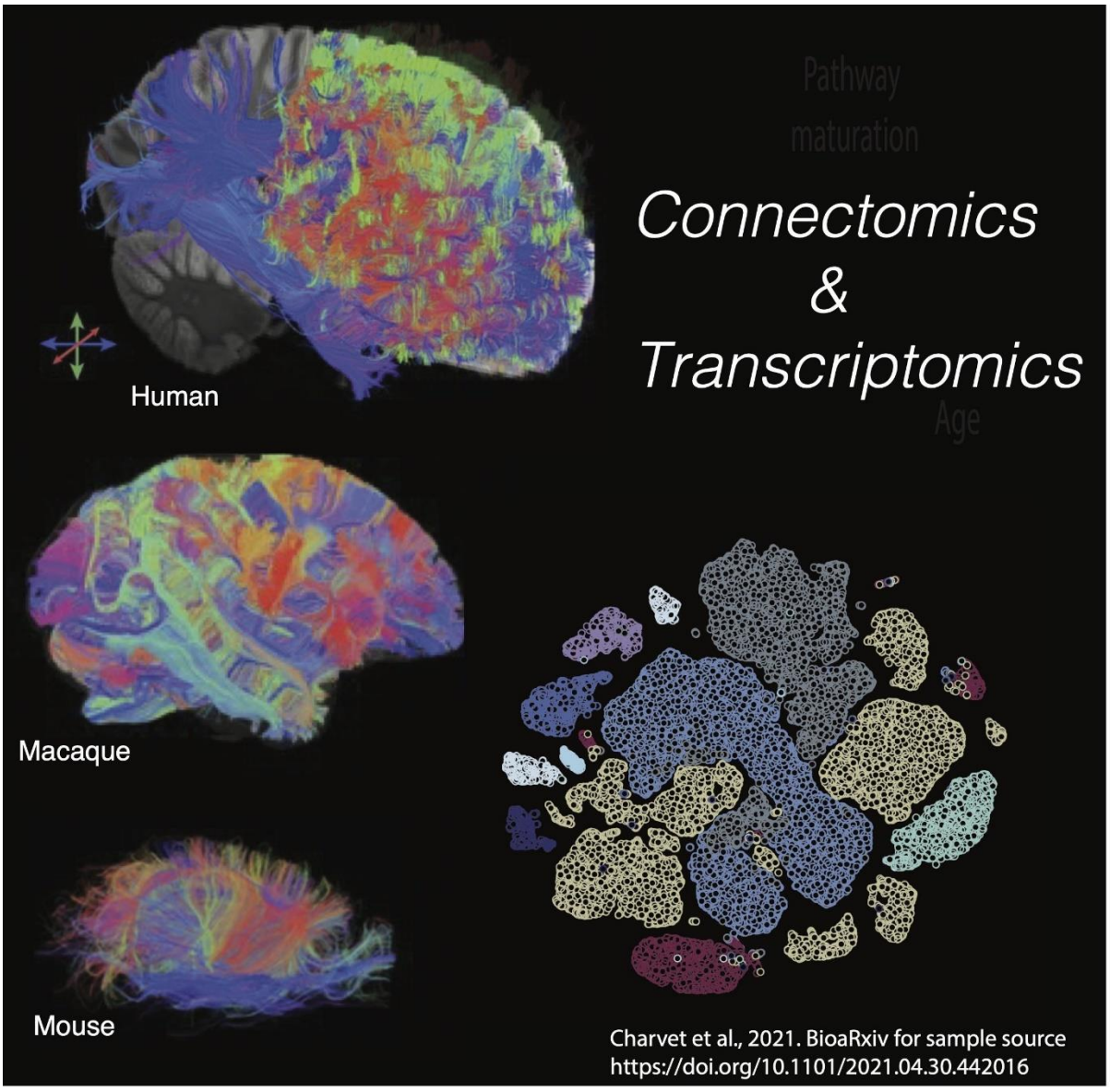
Topic: A.10. Development and Evolution

Support: NIGMS P20GM103446
NICHD R21HD101964
NIGMS 5P20GM103653

Title: Tracing modifications to cortical circuits in humans and non-human primates from high resolution connectomic, transcriptomic, and temporal dimensions

Authors: K. OFORI[1], C. BAUCUM[2], J. SUN[1], K. HEKMATYAR[3], M. S. MODRELL[1], B. EDLOW[4], A. VAN DER KOUWE[5], ***C. J. CHARVET**[1];
[1]Delaware State Univ., Dover, DE; [2]Bath Spa Univ., Bath Spa, United Kingdom; [3]Univ. of Delaware, Dover, DE; [4]Massachussetts Gen. Hosp., Boston, MA; [5]Martinos Ctr., Boston, MA

Abstract: The neural circuits that support human cognition are a topic of enduring interest. Yet, the lack of tools available to map human brain circuits has precluded our ability to trace the human and non-human primate connectome. We harnessed high-resolution connectomic, anatomic, and transcriptomic data to investigate the evolution and development of frontal cortex circuitry. We first developed enhanced tools to study the development of circuits across species. We used RNA sequencing data to find corresponding ages between humans and macaques. We also transcriptionally defined neural circuits by testing for associations between gene expression and white matter maturation. We then used these tools to test whether the timeline of frontal cortex circuit maturation is extended in humans in development. We also test for cross-species variation in adult frontal cortex circuits. We found that frontal cortex circuitry development is extended in primates, and concomitant with an expansion in cortico-cortical pathways compared with mice in adulthood. Importantly, we found that these parameters varied relatively little across humans and studied primates. These data identify a collection of conserved features in frontal cortex circuits across humans and Old World monkeys. We demonstrate that integrating transcriptional and connectomic data across temporal dimensions is a robust approach to trace the evolution of brain connectomics in primates.



Disclosures: K. Ofori: None. **C. Baucum:** None. **J. Sun:** None. **K. Hekmatyar:** None. **M.S. Modrell:** None. **B. Edlow:** None. **A. van der Kouwe:** None. **C.J. Charvet:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.02

Topic: A.10. Development and Evolution

Support: McDonnell Foundation grant 220020516
National Institute of Neurological Disorders and Stroke grant 1 F31 NS115242-01

Title: A common reference frame for comparing cortex-wide gene expression between species

Authors: *M. ENGLUND[1], S. JAMES[2], R. T. BOTTOM[3], K. J. HUFFMAN[4], S. WILSON[2], L. A. KRUBITZER[5];
[1]Univ. of California Davis, Davis, CA; [2]Univ. of Sheffield, Sheffield, United Kingdom; [3]Neurosci., [4]Psychology/Neuroscience, Univ. of California, Riverside, Riverside, CA; [5]UC Davis, Davis, CA

Abstract: Advances in sequencing techniques have made comparative studies of gene expression a recent focus for understanding evolutionary and developmental processes. These studies have provided invaluable information about species and cell-type specific gene expression in terms of differences in gene sequence and level of expression. Yet quantitative comparisons of spatial patterns of gene expression have been extremely limited due to a lack of robust methodology. Thus, we developed a set of algorithms for quantifying and comparing spatial gene expression within and across species, which we refer to as *Stalefish, The Spatial Analysis of Fluorescent (and non-fluorescent) In-Situ Hybridization*. Here, we apply these algorithms to quantify cortex-wide expression of Id2 and RZRb mRNA in early postnatal mice and voles to determine the extent to which spatial patterns of expression of these genes is conserved between species. We found that Id2 is moderately conserved across the neocortex in mice and voles, but the extent of this conservation varies by layer and area (e.g. high conservation in caudal neocortex). On the other hand, RZRb expression is tightly conserved in somatosensory cortices (S1 and S2) between species, while expression in visual (V1 and V2) and auditory (A1) areas vary. These differences may be the result of inbreeding in mice or products of natural independent evolution that have emerged in the 35 million since the last common ancestor. We believe scientists across fields will use this newly developed tool to quantify spatial patterns of gene expression (or other histological markers) to address differences between species, developmental time-points, or experimental conditions.

Disclosures: M. Englund: None. **S. James:** None. **R.T. Bottom:** None. **K.J. Huffman:** None. **S. Wilson:** None. **L.A. Krubitzer:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.03

Topic: A.10. Development and Evolution

Support: FONDECYT 1210471 to JCO

Millennium Nucleus of Ion Channels Associated Diseases (MiNICAD), Iniciativa Científica Milenio, Ministry of Economy, Development and Tourism from Chile to JCO
fondecyt 1191152 to MEA

Title: Evolutive origin of the RCOR genes and their interaction with LSD1

Authors: *M. OLIVARES COSTA[1], G. MERELLO OYARZUN[2], D. VERBEL VERGARA[1], M. ANDRÉS COKE[1], J. OPAZO[3];
[1]Univ. Católica, Santiago, Chile; [2]Columbia, New york, NY; [3]Univ. Austral, Valdivia, Chile

Abstract: Lysine-specific demethylase 1A (LSD1) is an epigenetic modifier that, together with the RCOR proteins, forms stable core subcomplexes recruited by several transcriptional repressors. The function and composition of the complex depends on the type of RCOR protein interacting with LSD1. Mice lacking the RCOR1 and RCOR2 proteins perinatally die and exhibit microcephaly. RCOR2 depletion resulted in reduced neural progenitor cell proliferation, neocortex thickness, and brain size. Little is known about the role of RCOR3, however, it has been proposed that it plays a compensatory role in the absence of RCOR1 or RCOR2. Due to the relevance of RCOR proteins and still lack of information about their potential different functions, we decided to evaluate the duplicative history of RCOR genes and analyze the evolutionary origin of their interaction with LSD1. Our phylogenetic analysis showed that jawed vertebrates are the only animals with three copies of the RCOR gene. We also found some species of vertebrates naturally lacking RCOR2. We have shown that the RCOR genes are onhologs because they are the product of the two duplications of the entire genome that occur in the ancestor of the vertebrates. We also predicted the ancestral sequence of the RCOR gene existing before the appearance of onhologs and evaluated a possible ancestral interaction with LSD1. Our data reveal the evolutionary origin of the RCOR-LSD1 complex and allow the selection of the best animal model for the study of the RCOR proteins.

Disclosures: M. Olivares Costa: None. **G. Merello Oyarzun:** None. **D. Verbel Vergara:** None. **M. Andrés Coke:** None. **J. Opazo:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.04

Topic: A.10. Development and Evolution

Support: NSF Grant R01
2CI Fellowship Center for Neuroinflammation and Cardiometabolic Diseases

Title: Neurodevelopmental implications of maternal and fetal inflammation at birth in the neonatal mouse

Authors: *J. M. GRAY[1], A. CASTILLO-RUIZ[1], K. MAJOR[1], T. A. HITE[2], N. G. FORGER[1]; [1]Georgia State Univ., Atlanta, GA; [2]Univ. of Texas, Austin, TX

Abstract: For placental mammals, birth is a remarkably inflammatory event for both mother and newborn, marked by a surge of inflammatory cytokines. In the present study, we investigated the role of maternal and fetal inflammation on perinatal neurodevelopment. Inflammatory responses within the body are largely mediated by Toll-like receptors (TLRs), almost all of which signal using adaptor protein myeloid differentiation factor 88 (MyD88) in both humans and murine species. The current investigation used MyD88 knockout mice (MyD88 KO), which globally lack MyD88-mediated inflammatory responses. To assess peripheral inflammation around the time of birth, we measured cytokine protein concentrations in fetal blood plasma, maternal blood plasma, and fetal milk spots using electrochemiluminescence multiplex assays, as well as in maternal uterine tissue using RT-qPCR. In fetal plasma, we observed significantly reduced levels of IL-6 (P=0.000), IL-8 (P=0.023), TNFα (P=0.003), and IL-10 (P=0.031) in MyD88 KO pups at embryonic day 18.5, regardless of maternal inflammatory status. However, IL-10 was significantly reduced (P=0.014) and IL-13 significantly increased (P=0.039) in all pups three hours postpartum born to MyD88 KO dams, regardless of inflammatory status, suggesting maternal inflammation drives offspring peripheral immune reactivity immediately after birth. We then assessed central inflammation by measuring cytokine expression in fetal hindbrain tissue using RT-qPCR and histological analysis of microglial density (Iba1) and cell death (αAC3), focusing on target brain regions sensitive to immune signaling. We observed that offspring born to MyD88 KO dams exhibit a striking reduction in cell death (PVN: P=0.010, CA1: P=0.008) and microglial labeling (PVN: P=0.020, CA1: P=0.000) at P0 in the paraventricular nucleus of the hypothalamus and CA1 Oriens of the hippocampus, indicating maternal inflammation drives perinatal microglial density and neuronal cell death. Overall, these data indicate that specific immune signals from both mother and newborn may act in tandem to drive neonatal inflammation and subsequent neurodevelopmental events throughout the perinatal period.

Disclosures: J.M. Gray: None. **A. Castillo-Ruiz:** None. **K. Major:** None. **T.A. Hite:** None. **N.G. Forger:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.05

Topic: A.10. Development and Evolution

Support: R01EY013528

R01EY019498
P30EY003176
K99EY030909

Title: Cellular mechanisms underlying embryonic retinal waves

Authors: *C. VOUFO[1], M. B. FELLER[2], A. TIRIAC[3];
[1]Univ. of California Berkeley, Oakland, CA; [3]Mol. and Cell Biol., [2]Univ. of California Berkeley, Berkeley, CA

Abstract: Spontaneous activity is a hallmark of developing neural systems. In the retina, spontaneous activity comes in the form of retinal waves persist from embryonic day 16 to postnatal day 14, when eye opening occurs. Though much is known about the mechanisms that mediate retinal waves during postnatal stages of development, little is known about the circuits that mediate embryonic waves. We used use two-photon calcium imaging to characterize retinal waves in the embryonic and pharmacology to assess the role of nAChR, gap junction antagonists and light stimulation on their properties. We find that waves are blocked by meclofenamic acid (50 µM) gap junction antagonist, and by the hexamethonium (100 µM), a general nAChR antagonist. Waves were still present though greatly diminished by the nAChR antagonist dihydro-ß-erythroidine (8 µM), which preferentially blocks nAChRs containing the ß2 subunit. Finally, we found that light stimulation of intrinsically photosensitive retinal ganglion cells did not generate a detectable light response but led to a small yet significant increase in wave frequency. Together these data suggest that a network consisting of ipRGCs, non-ipRGCs and cholinergic interneurons are involved in the generation Stage 1 waves.

Disclosures: C. Voufo: None. **M.B. Feller:** None. **A. Tiriac:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.06

Topic: A.10. Development and Evolution

Support: NIH Grant

Title: Cellular diversity in the Drosophila 3rdinstar larval ventral cord revealed by single-cell transcriptomics

Authors: *T. H. NGUYEN, R. VICIDOMINI, T. BRODY, S. D. CHOUDHURY, M. SERPE;
NICHD/NIH, bethesda, MD

Abstract: *Drosophila* ventral nerve cord (VNC), the fly equivalent of mammal the spinal cord, coordinates and integrates neural signaling from brain and periphery nervous system to produce a variety of locomotor outputs. During development, most of these locomotor outputs are executing by motorneurons (MNs), which innervate the body wall muscles. Our long-term goal is to understand how MNs develop plastic synapses, in particular what are the long-term transcriptional changes that return the circuits to steady-state functions. Here we use single cell RNA-seq to comprehensively describe the cellular diversity of *Drosophila* third instar larvae VNCs for the first time. We dissected third instar larvae VNCs, dissociated the cells and sequenced ~31,000 single cells. Using un-supervised clustering algorithms, we clustered these cells into distinct populations. We then assigned these populations to specific cell types using known markers. Through a series of reiterative processes, we first identified eight different glia subtypes, each with distinct metabolic pathways. Secondly, based on the expression of neuroblast genes, and the temporal determinant genes, we revealed a developmental trajectory leading from neural precursors to newborn neurons. We also detected novel differentially expresses genes along this trajectory. Thirdly, we identified over 40 types of clearly differentiated interneurons, each expressing unique combinations of transcription factors and neurotransmitters (*VGlut, Gad1, VAChT, DAT).* We found most of interneuron subtypes express multiple GPCRs and cell recognition molecules, suggesting that multiple sensory modalities converge onto single cells to elicit specific motor functions/behaviors. Finally, we identified a large motor neuron (MN) cluster. Within this MN population, we separated different types of MNs, including type I-b, type I-s, type II/octopaminergic and type-III/peptidergic. We also recognized different MN bundles, projecting dorsally, ventrally, or laterally. This single cell transcriptomic atlas provides a valuable resource for future studies of neurodevelopment and behavior.

Disclosures: T.H. Nguyen: None. **R. Vicidomini:** None. **T. Brody:** None. **S.D. Choudhury:** None. **M. Serpe:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.07

Topic: A.10. Development and Evolution

Title: Convergent evolution of alternative splicing in D2 dopamine receptor genes

Authors: *M. T. PEGLAR, K. J. FRYXELL;
George Mason Univ., Fairfax, VA

Abstract: D2 dopamine receptor mRNAs are alternatively spliced (in rodents and primates) by skipping exon 6 to produce the D2S isoform, or by including exon 6 to produce the D2L isoform.

The two D2 isoforms differ in their rates of cytoplasmic vesicular processing, calcium-mediated desensitization, and their involvement in pre- vs. post-synaptic dopamine signaling. Misregulation of D2 alternative splicing is associated with human psychiatric disorders including schizophrenia, major depression, and bipolar disorder. We have shown that the peptide sequence encoded by D2 exon 6 is unique to the D2 receptor, and is conserved from lamprey to human. We have further shown that exon 6 originated (in evolutionary history) after the duplication and divergence of D2/D4 receptors, but before the duplication and divergence of D2/D3 receptors. During subsequent evolution, exon 6 was highly conserved, and in fact it was the most conserved sequence in the third cytoplasmic loop of the D2 receptors of cyclostomes, sharks and fishes, none of which undergo alternative splicing of exon 6 in the brain. Nor does alternative splicing of exon 6 occur in relatively primitive amphibians (*Xenopus*, *Notophthalmus*), reptiles (turtles), birds (ostrich), or mammals (monotremes and marsupials). Alternative splicing of exon 6 does occur in the more derived species of amphibians (bullfrog), reptiles (lizards), birds (chickens, songbirds) and mammals (placental mammals). Based on these observations, we conclude that alternative splicing of D2 exon 6 evolved convergently at least four times, and has generally marked the more derived tetrapod lineages, as if increases in alternative splicing, and/or alternative splicing of this particular exon, were generally helpful in adapting to new environments. In contrast, D3 exon 6 has not been conserved, and the D3 gene itself has also been lost or partially deleted in some vertebrate species. In those species in which D3 exon 6 was still recognizably present, alternative splicing of D3 exon 6 was rare or absent. This provides an interesting contrast between a highly conserved D2 exon 6 that convergently evolved alternative mRNA splicing, versus a poorly conserved D3 exon 6 that did not.

Disclosures: M.T. Peglar: None. **K.J. Fryxell:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.08

Topic: A.10. Development and Evolution

Support: NSF PRFB 1812017
NIH U01 NS108637

Title: High-throughput single neuron transcriptomics and in situ hybridization chain reaction reveal neuronal expression of both candidate and novel genes in the brain of the nudibranch mollusc Berghia stephanieae

Authors: *M. D. RAMIREZ[1], T. N. BUI[2], P. S. KATZ[1];
[1]Univ. of Massachusetts Amherst, Amherst, MA; [2]MIT, Cambridge, MA

Abstract: Identifying neuronal cell types is essential for understanding neural circuits and brain function. Single cell transcriptomics allows molecular fingerprinting of neurons at an unprecedented depth, breadth, and throughput, and also works in species lacking genetic tools to label and identify neurons. We created unbiased single neuron transcriptomes using the 10x Genomics platform for ~2000 cells from the brain and rhinophore ganglia of the nudibranch, *Berghia stephanieae*. We performed standard clustering and differential gene expression analyses to find molecular markers for computational clusters of neurons with similar gene expression profiles. Many cluster markers were understudied or unannotated genes, emphasizing that much is still to be learned from "non-model" animals. CCWamide, a recently identified peptide in the annelid *Platynereis*, appeared as a top marker for one cluster. Although CCWamide had not been previously described in any mollusc, we localized its expression to a large number of neurons throughout the cerebral-pleural ganglia of *Berghia* using *in situ* hybridization chain reaction (HCR). Temptin, known only as a reproductive pheromone in *Aplysia*, was a top marker for neuron clusters from the rhinophore ganglia (RG). Another top cluster marker for RG neurons was the enzyme nitric oxide (NO) synthase. NO signaling is thought to be important in olfactory centers in other gastropods. Many markers had no similarity with annotated sequences in public databases (e.g. NCBI). However, we found orthologs of these genes in other molluscs, suggesting they are molluscan or perhaps spiralian novelties with unknown functions for the brain. Finally, we looked for candidate genes that are important for neuron function and identification. Numerous neuropeptides, including APGWamide, small cardioactive peptide and FMRFamide were found in the transcriptomes and labeled using HCR. These genes were often co-expressed in particular sets of peptidergic neurons, and were most often not expressed in the many neurons expressing the gene for choline acetyltransferase, the synthetic enzyme for acetylcholine. Transcription factors like sox2 and brn3 marked specific clusters that we localized to unique sets of spatially segregated neurons, a pattern unlike those we found for neurotransmitters. Overall, our single neuron dataset yielded both unbiased and candidate genes that marked specific sets of neurons in this new reference species. Molecular fingerprints for all cell types in the *Berghia* brain can be established based on combinations of gene expression for these markers, enabling us to create a brain-wide neuronal atlas.

Disclosures: M.D. Ramirez: None. **T.N. Bui:** None. **P.S. Katz:** None.

Poster

P079. Comparative Cellular and Molecular Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P079.09

Topic: A.03. Stem Cells and Reprogramming

Support: NIDA grant DA023999 to PR

Title: Cross-species comparison of the endocannabinoid signaling system in developing neocortex

Authors: *N. MICALI, Y. M. MOROZOV, P. RAKIC;
Yale Med. Sch., New Haven, CT

Abstract: The endocannabinoid signaling (ECS) pathway plays a central role in regulating fetal neurogenesis and neural circuit formation. Dysregulations of this signaling cascade in the neurogenic phase of human neocortex development, often due to marijuana consumption during pregnancy, might affect offspring's brain development and are associated with risk for neuropsychiatric disorders. Recent evidence from our lab indicates that timing of expression and sub-cellular distribution of the cannabinoid receptors in the neocortex are different in embryonic primates versus rodents. Thus, we need to understand how ECS' role in the early steps of corticogenesis varies across species to better define the effects of its alteration during human development. In the present study, we examined ECS during early corticogenesis in mouse, rhesus macaque and human. We used electron microscopy, cell imaging, transcriptomic and bio-informatics tools to define species-specific spatio-temporal dynamics of the ECS genes across the early phases of corticogenesis. Using *in vitro* models including human iPSC derived cortical brain organoids and monkey primary neural stem cells, we interrogated the role of candidate ECS genes across radial glial cell fate progression. We defined species-specific genetic and epigenetic regulation of the ECS genes during cortical area specification and in migrating cortical neurons. This comparative research approach provides new insights into the species-specific differences of ECS function in cortical development and enhance our understanding of the implications of prenatal cannabis consumption on the onset of neuropsychiatric disorders.

Disclosures: N. Micali: None. **Y.M. Morozov:** None. **P. Rakic:** None.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant MH105094
NARSAD Young Investigator Grant #28731

Title: Region-specific dopamine D2 autoreceptor signaling drives sex-biased behavioral changes in DAT Val559 mice

Authors: *A. STEWART[1,2], F. P. MAYER[1], R. GOWRISHANKAR[1], L. B. AREAL[1], S. E. STILLEY[1], A. BODINE[1], R. D. BLAKELY[1,2];
[1]Biomed. Sci., [2]Stiles-Nicholson Brain Inst., Florida Atlantic Univ., Jupiter, FL

Abstract: Virtually all neuropsychiatric disorders display sex bias, with the prevalence, age of onset, and clinical symptomology differing significantly between males and females. Here, we utilize mice expressing the dopamine (DA) transporter (DAT) Val559 variant, identified in patients with attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and bipolar disorder (BPD) to investigate the critical impact of sexual dimorphism in the architecture of the DA system in phenotypes driven by chronic DA dysfunction. In males, communication between DA D2 autoreceptors (D2ARs), responsible for a negative feedback loop that suppresses vesicular DA release following neuronal activation, and DAT, the primary determinant of DA clearance, modulates DA signaling specifically in the nigrostriatal circuit, heavily implicated in DA-driven locomotor behavior. This mechanism is hyperactive in DAT Val559 males, but, in females, perturbations in D2AR-DAT coupling are seen only in the reward-associated mesolimbic circuit. Furthermore, we observed a delay in DA clearance in the dorsal striatum of male DAT Val559 mice via *in vivo* high-speed chronoamperometry whereas DAT Val559 females displayed altered DA clearance in the ventral striatum. These sex differences in DA biology are predicted to translate into a differential impact of the mutation on DA-dependent behavioral phenotypes. Indeed, we found that male DAT Val559 mice exhibit decreased interaction in a social approach paradigm and increased wins in the tube test, whereas female DAT Val559 mice show no phenotype for these measures. In the light/dark transition test, female DAT Val559 animals spent less time in the light and had an increased latency to enter the light side, suggestive of heightened anxiety. Males, conversely, spent more time on the light side. Finally, male and female DAT Val559 mice demonstrated distinct deficits in memory domains, with DAT Val559 males exhibiting decreased spontaneous alternation in the Y Maze, whereas female DAT Val559 demonstrate reduced novel object recognition. Notably, both the altered spatial working memory of DAT Val559 males and the diminished novel object recognition phenotype of DAT Val559 females could be rescued through acute administration of the D2R antagonist sulpiride, providing clear evidence that ongoing, hyperactive D2R signaling contributes significantly to the sex-biased behavioral phenotypes of DAT Val559 mice. These efforts support a model whereby sexually-dimorphic elements underlying DA signaling establish differential behavioral trajectories and determine the penetrance of neuropsychiatric disease risk factors.

Disclosures: A. Stewart: None. **F.P. Mayer:** None. **R. Gowrishankar:** None. **L.B. Areal:** None. **S.E. Stilley:** None. **A. Bodine:** None. **R.D. Blakely:** None.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: FPM is supported by a Max Kade Fellowship (Max Kade Foundation NY, USA / Austrian Academy of Sciences)
AS is supported by a NARSAD Fellowship from BBRF
NIH Award MH105094 to RDB

Title: Impact of functional dopamine transporter coding variation on naturalistic reward-induced dopamine release in the nucleus accumbens in vivo as monitored by fiber photometry

Authors: *F. P. MAYER[1], H. PHELPS[1], H. IWAMOTO[2], A. STEWART[2], M. RABIL[1], R. D. BLAKELY[2];
[1]Dept. of Biomed. Sci., [2]Dept. of Biomed. Science, Brain Inst., Florida Atlantic Univ., Jupiter, FL

Abstract: Dopamine (DA) is a critical modulator of a wide range of processes, ranging from locomotor activity, reward and response to novelty, mood and attention. Consequently, dysfunction in DA signalling has been linked to multiple disorders that feature deficits in these processes, including attention-deficit/hyperactivity disorder (ADHD), schizophrenia, bipolar disorder and autism. At a synaptic level, the local availability of DA is dictated by coordinated processes of DA release and clearance, with the latter process overseen by the presynaptic, cocaine- and amphetamine-sensitive DA transporter (DAT, *SLC6A4*). Our lab identified the DAT Val559 coding variant in subjects diagnosed with ADHD, with others demonstrating its presence in individuals with autism or bipolar disorder. Although DAT Val559 displays normal DA uptake function in transfected cells and in synaptosomes from DAT Val559 knock-in mice, we have presented evidence that the variant supports an anomalous, DAT-dependent DA efflux process that *in vivo* alters extracellular DA levels in the striatum, leading to constitutive activation of presynaptic D2-type autoreceptors, increased surface expression of leak-prone transporters and diminished vesicular DA release. At a behavioral level, these effects alter locomotor responses to psychostimulants, waiting impulsivity and enhanced motivation for reward as well as reduced working memory. We have shown recently (Mayer FP et al., 2021) that genetically-encoded DA sensors monitored by fiber photometry *in vivo* reveal a striking pattern of DA release in the nucleus accumbens (NAc) when mice are moved from a standard testing chamber back to their home cage, providing an opportunity to monitor the impact of the DAT Val559 variant on the DAergic responses to a naturalistic reward. Our studies indicate that wild-type 9-week old male mice demonstrate the expected rise in DA levels following their transfer from the recording chamber to their home cage. In contrast, male Val559 littermates display a reduced DA rise, in both amplitude and duration. These findings provide direct evidence that the ADHD-associated Val559 variant impacts the molecular dynamics of behaviorally-induced DA release *in vivo* in the NAc. These observations may be of clinical relevance in providing further opportunities to use the paradigm for the development of treatments for disorders that feature perturbed DA signalling.

Disclosures: F.P. Mayer: None. **H. Phelps:** None. **H. Iwamoto:** None. **A. Stewart:** None. **M. Rabil:** None. **R.D. Blakely:** None.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: J.T.Y. is supported by intramural funding through BYU and the American Osteopathic Association

Title: Exploration of michaelis-menten kinetics of the dopamine transporter in the striatum using iontophoresis.

Authors: *J. P. W. RONSTRÖM[1], A. EVERETT[2], E. NILSSON[2], B. TOLLMAN[2], A. ALLRED[2], B. BRODIE[2], K. ROBINSON[2], M. BURRIS[3], J. T. YORGASON[1];
[2]Neurosci., [3]Dept. of Neurosci., [1]Brigham Young Univ., Provo, UT

Abstract: Exploration of Michaelis-Menten kinetics of the dopamine transporter in the striatum using iontophoresis. The main objective of this study is to investigate the Michaelis-Menten (MM) kinetics for the dopamine transporter (DAT) in the striatum and the effect of psychostimulants on DAT trafficking. Traditionally, the Michaelis-Menten (MM) enzymatic model is used to determine the maximal rate of uptake (V_{max}) of dopamine (DA) by the DAT as well as provide information on the enzymatic binding activity (K_m). The MM parameters for the DAT have been examined and characterized previously, albeit under non-physiological conditions that may not be reflective of actual DAT activity. First, previous experiments determining MM K_m parameters have used homogenized tissue. The preparation of homogenized tissue is crude and disrupts ion gradients, intracellular organelles, such as mitochondria, and overall cellular function. Thus, previous estimates of MM parameters based on these conditions may be adversely affected by these non-physiological conditions. Second, voltammetry studies measuring evoked dopamine release have assumed DAT saturation for determining V_{max} without proving saturation under electrically stimulated release conditions. The assumption for DAT saturation is again based partly on K_m values from homogenized tissue studies, which estimate K_m values at ~160-200 nM. Furthermore, DAT V_{max} values from MM modeling studies correlate with release amplitude, suggesting that DATs are undersaturated and MM conditions are being violated for some of these previous studies. The validity of these parameters was investigated using exogenously application of DA (iontophoresis and puff application) in the striatum in adolescent mice. The DAT was saturated by applying exogenous DA with increasing concentrations, while measuring uptake. The V_{max} and K_m parameters were then calculated from these experiments. To quantify the effect of psychostimulants on these MM parameters, the same

procedure was conducted using increasing concentrations of cocaine and GBR12909—two drugs known to inhibit DAT activity.

Disclosures: J.P.W. Ronström: A. Employment/Salary (full or part-time):; Brigham Young University Neuroscience program. **A. Everett:** A. Employment/Salary (full or part-time):; Brigham Young University Neuroscience program. **E. Nilsson:** A. Employment/Salary (full or part-time):; Brigham Young University Neuroscience program. **B. Tollman:** None. **A. Allred:** None. **B. Brodie:** None. **K. Robinson:** None. **M. Burris:** None. **J.T. Yorgason:** A. Employment/Salary (full or part-time):; Brigham Young Unvirsity Cellular biology and pshysiology.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant DA035714
NIH Grant DA041932

Title: Characterizing the pharmacological profile of novel allosteric ligands of human dopamine transport: Potential therapy for HIV-1 associated neurocognitive disorders

Authors: *A. JIMENEZ-TORRES[1], O. MOUKHA-CHAFIQ[2], P. K. M. VENUKADASULA[2], R. HUNTER[2], T. NGUYEN[2], M. YATCHANG[2], S. ZHANG[2], C. E. AUGELLI-SZAFRAN[2], J. ZHU[1];
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Abstract: HIV-1 regulatory protein, transactivator of transcription (Tat), and cocaine synergistically dysregulate synaptic dopamine (DA) transmission by directly inhibiting DA transporter (DAT) activity, ultimately leading to dopaminergic neuron damage, which has been implicated as a mediated factor for developing HIV-1 associated neurocognitive disorders (HAND). Given the continuing presence of Tat protein in HIV patients with antiretroviral therapy, there is a pressing need to develop neuroprotective agents for preventing the progression of HAND. We have demonstrated that Tat-induced inhibition of DAT is mediated by the binding of Tat to allosteric binding site(s) on DAT and not by interacting with the DA uptake site. This provides a basis for developing compounds to attenuate Tat binding to DAT by an allosteric mechanism while having minimal influence on physiological DA transport. We have developed a series of quinazoline-based compounds that display allosteric modulatory effects at DAT, in which SRI-32743, as a novel candidate, attenuates Tat-induced inhibition of DAT-mediated DA

uptake *in vitro* and alleviates Tat-augmented cocaine reward. This study determined the structure-activity relationships of 112 analogs of SRI-32743 for identifying compounds with a balanced allosteric DAT interaction profile along with improved physicochemical and drug-like properties. SRI-32743 analogs were designed by *in silico* analysis in virtual libraries and retained the basic 7-methylimidazo[1,2-a]pyridin-6-yl moiety that is attached to the 2-position of the quinazoline ring system. Initially, we conducted the pharmacological screening for the synthesized analogs of SRI-32743 at three concentrations (10 nM and 1 and 10 µM) by assessing their inhibitory effects on [^{3}H]DA uptake and [^{3}H]WIN35-428 binding in CHO-K1 cells expressing wild-type human DAT. Compounds with over 50% inhibition at 10 µM were subjected to evaluating inhibition potencies (IC_{50} values) for DAT function and binding. Here we present the pharmacological profiles of 12 selected analogs of SRI-32743 (IC_{50} values of 3.55-17.87 µM for inhibiting [^{3}H]DA uptake and IC_{50} values of 1.29 - 35.88 µM for inhibiting [^{3}H]WIN35-428 binding) versus SRI-32743 with IC_{50} values 9.86 and 4.59 µM for inhibiting DA uptake and DAT binding, respectively. Determination of the allosteric modulatory function and capability in attenuation of Tat's effect of these selected analogs will further reveal potential for a therapeutic application in HAND.

Disclosures: A. Jimenez-Torres: None. **O. Moukha-Chafiq:** None. **P.K.M. Venukadasula:** None. **R. Hunter:** None. **T. Nguyen:** None. **M. Yatchang:** None. **S. Zhang:** None. **C.E. Augelli-Szafran:** None. **J. Zhu:** None.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.05

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: J.T.Y. is supported by intramural funding through BYU and the American Osteopathic Association
C.A.S. is supported by a NIH grant R00 DA04510 (NIDA)
R.A.E. is supported by a NIH grant R01 DA031900 (NIDA)

Title: Modeling aspects of dopamine release and reuptake using fast scan cyclic voltammetry

Authors: A. C. EVERETT[1], B. E. GRAUL[1], D. B. WATTS[1], R. A. ESPAÑA[2], C. A. SICILIANO[3], ***J. T. YORGASON**[1];
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Abstract: Fast-scan cyclic voltammetry (FSCV) is a highly effective electrochemistry technique for measuring rapid dopamine (DA) release and clearance, particularly in the nucleus accumbens

and dorsal striatum. The DA terminals in these areas are abundant, and DA concentrations are heavily regulated by the dopamine transporter (DAT). Historically, the Michaelis-Menten model has been a standard in measuring DAT enzymatic kinetics. However, this approach has historically used experimenter-fitted modeling and is therefore extremely subject to experimenter bias. This study presents a novel, unbiased analysis technique using the first (velocity) and second (acceleration) derivatives of evoked DA signals. Maximal upwards derivatives predict changes in DA release due to D_2 and $GABA_B$ agonist-mediated DA release inhibition. Maximal downwards derivatives are indicative of DA reuptake that is relatable to the Michaelis-Menten V_{max}, an indirect measure of DAT activity based on the number of expressed DATs. When evoked DA release in DATKO mice was subtracted from DA release in WT mice, DAT kinetics were accurately modeled. Increasingly negative velocities correlated with higher DAT activity. Critically, the Michaelis-Menten model is dependent upon an enzyme-specific constant, precluding its usage in DATKO (or similar enzymatic knockout) mice. In addition to DATKO data, the effect of two competitive, high-affinity DAT inhibitors (PTT and WF23) and the lower affinity inhibitor cocaine were analyzed to study relative effects on velocity and acceleration measures. All three inhibitors had clear effects on increasing upward velocity and decreasing downward velocity, with greatest effects on downward velocity at highest concentrations. These tools provide opportunities to study DA kinetics in situations previously inaccessible using current methodologies.

Disclosures: A.C. Everett: A. Employment/Salary (full or part-time):; Brigham Young University. **B.E. Graul:** A. Employment/Salary (full or part-time):; Brigham Young University. **D.B. Watts:** None. **R.A. España:** A. Employment/Salary (full or part-time):; Drexel University. **C.A. Siciliano:** A. Employment/Salary (full or part-time):; Vanderbilt University. **J.T. Yorgason:** A. Employment/Salary (full or part-time):; Brigham Young University.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.06

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIMH grant MH095044 (RDB)
Stiles-Nicholson Foundation ASCEND Postdoctoral Fellowship (LBA)
Max Kade Postdoctoral Fellowship (FPM)

Title: Region and Sex-Dependent Developmental Plasticity of Mouse Serotonergic Innervation Arising from the ADHD and Autism-Associated DAT Val559 Mutation

Authors: *L. B. AREAL[1], F. P. MAYER[1], A. STEWART[1], R. D. BLAKELY[2];
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Abstract: Disruptions in dopamine (DA) and serotonin (5-HT) signaling are believed to contribute to multiple neuropsychiatric disorders. The rare DA transporter (DAT) coding variant Val559, previously identified in subjects with Attention-Deficit/Hyperactivity Disorder (ADHD), autism spectrum disorder and bipolar disorder, promotes anomalous, DAT-dependent DA efflux. Mice engineered to express the Val559 mutation exhibit altered responses to the psychostimulants amphetamine and cocaine and display heightened impulsivity and reward motivation. We have previously shown that the loss of locomotor responses to cocaine in DAT Val559 mice involves a serotonergic plasticity since genetic elimination of cocaine binding at SERT restores cocaine actions, and locomotor activation occurs when animals are treated with a cocaine analog with reduced SERT action. Since increased levels of 5-HT have been found in the striatum and cortex of DAT Val559 mice, we hypothesized that the Val559 mutation induces a compensatory neuroplasticity that involves changes in serotonergic axon density. Immunofluorescent staining for 5-HT was performed with brain sections from DAT Val559 and littermate WT mice at 3 weeks and 7 weeks of age. 3D reconstruction and fiber tracing analysis revealed an increase in serotonergic axonal branching, length and volume in the dorsal striatum of 7-week-old DAT Val559 male mice, compared to WT. These changes were not observed in the dorsal striatum of DAT Val559 mice at 3 weeks of age, nor were changes found in the ventral striatum of 3- or 7-week-old mice. Consistent with these findings, microdialysis experiments showed increased baseline 5-HT extracellular levels in the dorsal striatum of DAT Val559 mice and increased 5-HT release induced by d-fenfluramine was revealed by fiber photometry in this region. Interestingly, 7-week-old female DAT Val559 mice showed increased 5-HT axon density in the medial prefrontal cortex, whereas no change was seen in males in this region. Consistent with this observation, head twitch responses induced by 2,5-dimethoxy-4-iodoamphetamine (DOI), known to be reliant on cortical 5-HT signaling, was observed in females but not in males. The present data reveals a functional, developmental and region-dependent alteration of serotonergic innervation arising in the context of the DAT Val559 mutation, pointing to the adolescent period as a critical developmental stage for the emergence of DA-dependent serotonergic plasticities. Furthermore, these results suggest that an altered interplay between DA and 5-HT during development may contribute to the sex-dependence of risk and trajectory of neuropsychiatric disorders.

Disclosures: L.B. Areal: None. **F.P. Mayer:** None. **A. Stewart:** None. **R.D. Blakely:** None.

Poster

P082. Dopamine Transport and Signaling

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P082.07

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Voluntary aerobic exercise protects against chronic ethanol-induced changes to kappa-opioid receptors

Authors: *D. Z. OTTESON[1,2], J. BRUNDAGE[1], B. M. WILLIAMS[1], J. A. SAUNDERS[1], J. T. YORGASON[1], K. B. BILLS[2,1], S. C. STEFFENSEN[1];
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Abstract: Exercise has long been prescribed as adjunctive treatment for alcohol-use disorder (AUD) despite the underlying mechanisms remaining largely uncharacterized. Mu-opioid receptors (MORs), delta-opioid receptors (DORs) and kappa-opioid receptors (KORs) are the three types of opioid receptors expressed in the brain which are part of the mesolimbic dopamine system and have long been thought to drive alcohol withdrawal symptoms. They attenuate dopamine (DA) release in the nucleus accumbens (NAc) and are known to sensitize following chronic ethanol exposure. The objective of this study was to evaluate the effect of exercise in conjunction with chronic ethanol on opioid receptor expression and DA release in the NAc. To answer this question, we used fast scan-cyclic voltammetry (FSCV), immunohistochemistry (IHC) and a behavioral assay, specifically the drinking-in-the-dark (DID) paradigm, which measures self-administration of ethanol in mice. Using FSCV, we found that a regiment of aerobic exercise was protective against ethanol-induced sensitization of KORs compared to non-exercised controls. Exercised mice displayed significantly lowered DA release upon exposure to KOR agonist u50488 using FSCV and reduced levels of KOR expression on DA neurons in the ventral tegmental area and NAc using IHC. Additionally, we found that aerobic exercise had no significant effect on DA release following superfusion of MOR agonist DAMGO; however aerobic exercise increased DA release following superfusion of DOR agonist DPDPE. Using IHC, we found that aerobic exercise was protective against ethanol-induced sensitization of DORs compared to non-exercised controls. Furthermore, in our DID model, aerobic exercise decreased seeking behavior compared to non-exercised controls. The findings in this study confirm that exercise is an effective adjunctive treatment for AUD and helps to illuminate the underlying mechanisms behind the protective effects of exercise on ethanol-induced changes to KORs.

Disclosures: D.Z. Otteson: None. **J. Brundage:** None. **B.M. Williams:** None. **J.A. Saunders:** None. **J.T. Yorgason:** None. **K.B. Bills:** None. **S.C. Steffensen:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.01

Topic: B.02. Ligand-Gated Ion Channels

Support: Foundation for a Smoke Free World (FSFW)

Title: SARS-CoV 2 spike protein-derived peptides inhibit the alpha 7 nicotinic acetylcholine receptor and are counteracted by a positive allosteric modulators of alpha 7

Authors: *J. FARLEY, J. B. ANDERSON;
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Abstract: Based on clinical observations of low prevalence of smoking among hospitalized COVID-19 patients and identification of snake "toxin-like" amino acid (aa) sequences in the spike protein of SARS-CoV-2, Changeux et al 2020 *C R Biol* and Farselinos et al 2020 *J Toxicol* (C & F) proposed that the hyper-inflammation of severe COVID-19 reflects dysfunction of the "cholinergic anti-inflammatory pathway" (CAP). In seminal CAP work, Tracey et al (2002, 2012) found that vagal nerve release of acetylcholine (ACh) suppressed activation of NF-κB and production of proinflammatory cytokines in α7 nicotinic ACh receptor- (α7 nAChR-) expressing macrophages. C & F proposed that SARS-CoV-2 spike protein (allowing viral entry into cells via ACE2) also binds and inhibits several nAChRs. The most critical of these in peripheral immune cells may be α7 Rs, activated on macrophages and T-cells by ACh, leading to decreased release of inflammatory cytokines. An important implication *ex hypothesi* that nAChRs are targets for spike proteins is that nAChR-activators/modulators may be useful in battling COVID-19. But we are unaware of any direct evidence that spike protein(s) bind and inhibit activation of α7, or other nAChRs. We tested a recombinant SARS CoV-2 (L strain) spike protein construct that included most of the S1 region (Gln14-Arg685 and a c-mFc tag). *Xenopus* oocytes expressing murine α7 R and exposed to this protein (100 nM concentration, 5-8 m) showed ~73% inhibition of peak α7-current evoked by 500 μM ACh. dH2O-exposed control oocytes (n=9) had a peak current (mean ± sem) of 262.4 ± 88.3 nA; the value for spike-protein treated cells (n=8) was 71.4 ± 32.3 nA ($p < .05$). Subsequent tests of 1 nM of this protein also produced significant ~59% inhibition of peak current. Controls (n=8) had peak current of 2565.8 ± 1100.6 nA; the value for S1 protein-treated cells (n=8) was 1064.7 ± 430.0 nA ($p < .005$). We also found that ~5 m exposure to 100 uM genistein (gen), a positive allosteric modulator of α7, counteracted inhibition by 1 nM spike protein. Gen-alone treated cells (n=3) had a peak current of 5553.4 ± 2287.4 nA. Cells exposed to both 1 nM S1-protein + gen (n=6) had peak current of 3870.7 ± 552.0 nA. ANOVA for these 4 treatments [$F(3,19) = 9.3$, $p < .0005$] and Tukey HSD tests indicated significant differences between: control vs. gen-exposed cells, and S1-protein exposed cells vs. S1-protein + gen exposed cells. Notably, gen abolished the S1-protein inhibition of α7 current. Tests of additional S1-protein constructs (e.g., Val16-Gln677) suggest that aa 375-390 are critical for inhibition. As C & F noted, spike protein-nAChR interactions may contribute to COVID-19 pathophysiology in immune and nervous systems.

Disclosures: J. Farley: None. **J.B. Anderson:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.02

Topic: B.02. Ligand-Gated Ion Channels

Title: Nicotine enhances SARS-CoV-2 glycoprotein functional modulation of the alpha7 nicotinic acetylcholine receptor

Authors: *B. C. V. O'BRIEN, M. M. WELTZIN;
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Abstract: SARS-CoV-2 has been proven to enter cells via targeting of its spike glycoprotein to angiotensin converting enzyme 2 (ACE2). Interestingly, the SARS-CoV-2 glycoprotein contains a region with similar sequence homology to other neurotropic virus glycoproteins, including rabies and HIV, and the neurotoxin domain found in snake toxins and protoxins. Such toxins and viral glycoproteins have been proven to interact with nicotinic acetylcholine receptors (nAChRs). Data collected from multiple countries has indicated smoking as a factor to decrease risk of SARS-CoV-2 related hospitalization, and that smokers who are admitted tend to experience increased disease progression and worse outcomes. We hypothesize that the SARS-CoV-2 spike glycoprotein neurotoxin domain interacts with nAChR subtypes, and that nicotine may enhance this interaction in a concentration dependent manner. To test our hypothesis, in *Xenopus laevis* oocytes, we expressed the most common nAChR subtypes, and their associated isoforms, that are potentially involved in SARS-CoV-2 infection. Using two-electrode voltage clamp electrophysiology, concentration response profiles were generated to test how the glycoprotein peptide affected response to acetylcholine in the absence and presence of physiologically relevant concentrations of nicotine. Our results show that the SARS-CoV-2 glycoprotein peptide interacts with nAChRs in a subtype specific manner. Further, the SARS-CoV-2 glycoprotein peptide has a bimodal effect on alpha7 nAChRs, including potentiation of agonist responses at low concentrations of peptide, and inhibition at higher peptide concentrations. Pre-incubation with nicotine at concentrations found in smokers blood enhanced the amount of potentiation and inhibition induced by the SARS-CoV-2 glycoprotein peptide. Our findings provide clues as to the reason for lower prevalence of hospitalized smokers as well as their tendency to see worse outcomes once hospitalized. In addition, we have identified the a7 nAChR, which is found in the brain, immune cells, and in respiratory epithelial cells, as a potential new therapeutic target for the treatment of SARS-CoV-2.

Disclosures: B.C.V. O'Brien: None. **M.M. Weltzin:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.03

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant R15NS093590
NIH grant R01GM108580
NIH grant R35GM140947

Title: Cmpi is a high-affinity, high-efficacy agonist at the α4:α4 acetylcholine binding site of (α4)3(β2)2 nicotinic acetylcholine receptor.

Authors: F. DEBA[1], E. PEREDIA[1], K. MUNOZ[2], G. AKK[3], ***A. K. HAMOUDA**[1];
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Abstract: The extracellular domain of the (α4)3(β2)2 nicotinic acetylcholine receptor (nAChRs) harbors two high-affinity ACh binding sites located in the two α4:β2 intersubunit interfaces and a low-affinity ACh binding sites located in the α4:α4 intersubunit interface. In this study, we used ACh, cytisine, and nicotine that bind at both the α4:α4 and α4:β2 interfaces, TC-2559 as a ligand that binds at the α4:β2 but not the α4:α4 interface, and CMPI as a ligand that binds at the α4:α4 but not the α4:β2 interface to investigate the interplay between these two classes of binding sites and their effect on channel gating. We recorded whole-cell currents of *Xenopus laevis* oocytes expressing (α4)3(β2)2 nAChR elicited in response to application of these ligand (alone or in combination at various concentrations). The electrophysiological data were analyzed in the framework of a modified Monod-Wyman-Changeux (MWC) allosteric activation model. Our results indicate that CMPI is a high-affinity, high-efficacy agonist at the α4:α4 ACh binding site. CMPI binds at the α4:α4 interface with a higher affinity than ACh, cytisine or nicotine. In addition, the gating efficacy of CMPI at the α4:α4 site is equivalent to that of ACh, while CMPI weak direct activation is accounted for by a single binding site mediating its action. As such, CMPI enhances channel gating triggered by agonist occupancy at the α4:β2 ACh binding sites by providing ligand occupancy at the α4:α4 ACh binding site which is otherwise not occupied at all by agonist or only occupied at very high agonist concentrations. The data presented here enhance our understanding of the functional contributions of ligand binding at the “non-canonical” α4:α4 subunit interface to (α4)3(β2)2 nAChR channel gating.

Disclosures: F. Deba: None. **E. Peredia:** None. **K. Munoz:** None. **G. Akk:** None. **A.K. Hamouda:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.04

Topic: B.02. Ligand-Gated Ion Channels

Support: GM136430
GM103801

Title: Ligand-binding promotes functional expression of human α9/α10 nicotinic acetylcholine receptors in Xenopus laevis oocytes

Authors: *A. J. HONE[1,2], J. M. MCINTOSH[1,2];
[1]Sch. of Biol. Sci., Univ. of Utah, Salt Lake City, UT; [2]Veterans Affiars Med. Ctr., Salt Lake City, UT

Abstract: Nicotinic acetylcholine receptors (nAChRs) containing α9 and α10 subunits have been implicated in neuropathic pain and inflammation. Separately, these receptors are expressed in hair cells of the cochlea where they modulate auditory processing. Cone snails are venomous marine mollusks that hunt worms, other mollusks, and fish. Some cone snails have α-conopeptides as components of their venom and may use these peptides to disorient fish prey by inhibiting α9 nAChRs expressed by hair cells of the lateral line sensory organ. α-Conopeptides are useful pharmacological tools for characterizing the expression of nAChRs in neurons and other cell types. Unfortunately, poor heterologous expression has hindered in depth pharmacological characterization of human α9/α10 subtypes. However, recently it was shown that transmembrane proteins of the inner ear, involved in human deafness, in concert with nAChR ligands are capable of promoting the expression of α9α10 nAChRs. *Xenopus* oocytes are routinely used for expressing nAChRs; previous reports have shown acetylcholine elicits robust functional responses when cRNAs for both α9 and α10 subunits are co-injected but little or no response when only α9 subunit cRNA is injected and no response when only α10 subunit cRNA is injected. We used a battery of α-conopeptides and other nAChR ligands to further examine the effects of ligand-binding on the functional expression of α9/α10 receptors and show that robust expression can be obtained by pre-exposure to select compounds. Furthermore, differential ligand effects were found for oocytes expressing α9 homomers vs. those expressing α9α10 heteromers. The results of these studies will help facilitate pharmacological characterization and drug discovery of therapeutic ligands for α9/α10 nAChRs.

Disclosures: A.J. Hone: None. **J.M. McIntosh:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.05

Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC Discovery Grant RGPIN-2019-04989
NSERC Discovery Grant RGPIN-2018-04699

Title: Functional expression of nicotinic receptor isoforms on distinct categories of pyramidal neurons within layer 5 of the mouse medial prefrontal cortex

Authors: *A. PATEL[1], A. NGUYEN[1], P. PALETTA[2], E. CHOLERIS[2], C. D. BAILEY[1];
[1]Dept. of Biomed. Sci., [2]Dept. of Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: Nicotinic acetylcholine signalling within the rodent medial prefrontal cortex (mPFC) is important for higher-order cognitive functions. Nicotinic receptors (nAChRs) are present on pyramidal neurons within layer 5 of the mPFC. These neurons form the primary signalling output from the mPFC and may be categorized as either regular-firing or initial burst-firing, based on threshold responses to positive current injection. We sought to complete a detailed characterization of nAChR function and neuron morphology for these neuron categories in young postnatal mice (P15-20) of both sexes, using whole-cell electrophysiology combined with biocytin filling and retrograde labelling. Pharmacological experiments using receptor isoform-selective antagonists identified a striking specificity for the postsynaptic function of the two major nAChR isoforms, in that regular-firing neurons respond to the α4β2* nAChR exclusively whereas burst-firing neurons respond to either the α7 nAChR exclusively or to a combination of α4β2* and α7 nAChRs. Comparison of the neuron categories determined that regular-firing neurons are more excitable due to their greater membrane resistance and smaller post-firing afterhyperpolarization, and receive a lower frequency of spontaneous excitatory postsynaptic currents (EPSCs). Morphological analysis determined that regular-firing neurons have more apical dendrite matter distal to the soma and more basal dendrite matter proximal to the soma. Retrograde labelling revealed that projections to the thalamus are made exclusively from regular-firing neurons that contain the α4β2* nAChR, projections to the contralateral mPFC are made exclusively from burst-firing neurons that contain the α7 nAChR only, and projections to the nucleus accumbens are made exclusively from burst-firing neurons that contain both α4β2* and α7 nAChRs. These novel findings suggest a detailed receptor isoform-based mechanism by which nicotinic signalling is regulated within the main output layer of young postnatal mPFC, which may influence the development and function of cognitive circuits involving this brain region.

Disclosures: A. Patel: None. **A. Nguyen:** None. **P. Paletta:** None. **E. Choleris:** None. **C.D. Bailey:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.06

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825
PJT-153101 (EKL)
CIHR CGS D (S POWER)

Title: Functional changes in prefrontal cholinergic synapses during adulthood: an optophysiological investigation

Authors: *S. K. POWER[1], S. VENKATESAN[1], E. K. LAMBE[1,2,3];
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Abstract: Cholinergic modulation of the prefrontal cortex is essential for attention and executive function. During attention, basal forebrain neurons projecting to the prefrontal cortex release acetylcholine, exciting deep-layer pyramidal neurons. This cholinergic synapse is complex and has been hypothesized to change with age, though this has not been studied functionally. Broad cholinergic alterations have been reported with human aging, such as decreases in nicotinic binding and cholinergic enzymes and fibers. These differences are hypothesized to underlie behavioural declines in attention, cue detection, and executive function in aging adults; however, their integrated impact on cortical cholinergic signalling is not well understood. Here, we use optogenetics and whole cell electrophysiology to investigate functional properties of prefrontal cholinergic synapses across a broad age range in adulthood. We use brain slices from transgenic mice expressing excitatory opsin in cholinergic basal forebrain neurons whose axons project to the cortex and release acetylcholine when stimulated with light. We demonstrate that pyramidal neurons in the adult brain show age-related changes in the properties of their responses to optogenetically-released acetylcholine. Direct excitation of cholinergic receptors on pyramidal neurons declines with age, with deficits arising most noticeably in the nicotinic component of these responses. Ongoing experiments are probing the age-sensitivity or resilience of specific presynaptic and postsynaptic components of the synapse with an eye to improving treatment targets. To this end, we are investigating age-dependence in the sensitivity of functional cholinergic signalling to specific allosteric modulators and other interventions. The goal of understanding the impact of age on synaptic mechanisms of cholinergic transmission is to improve treatments for cognitive impairment and better understanding of cognitive resilience.

Disclosures: S.K. Power: None. **S. Venkatesan:** None. **E.K. Lambe:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.07

Topic: B.02. Ligand-Gated Ion Channels

Support: Consulting for a nonprofit foundation that wishes to be anonymous

Title: Speculation on how RIC3 assists alpha7 nicotinic receptor pentameric assembly

Authors: R. H. LORING[1], K. AGARWAL[2], M. GUPTA[2];
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Abstract: We know little about how transmembrane proteins fold and assemble even though they form the majority of neurotransmitter receptors. Alpha7 nicotinic acetylcholine receptors (a7R) are unique (so far) among Cys-loop receptors in having a "latch" (Noviello et al., Cell, 184:1, 2021), an amphipathic alpha-helix (AH) at the C-terminal which is preceded by a Ser-Ala-Pro (SAP) motif. Although the latch sequence is **not** conserved in invertebrates, the SAP motif **is** conserved among a7-like receptors including *C. elegans* ACR-16. RIC3 (Resistance to inhibitors of cholinesterase 3) assists pentameric assembly of a7-like receptors across species evolution including ACR-16. Recent structural data suggest a groove forms within single a7R subunits between the latch, transmembrane (TM) domain 4 (including the helix that starts in the cytoplasmic loop-the CyM4AH), and another amphipathic helix located after M3 on the cytoplasmic side of the membrane (the cytoplasmic helix or CAH). **Hypothesis:** The structure of RIC3 is unknown but includes at least one TM helix which possibly fits into the groove of single a7R subunits, which we hypothesize aids in the assembly of a7Rs. One unknown is whether the rest of the cytoplasmic loop linking CAH to CyM4AH covers the groove, but this loop is not well resolved for any Cys-loop receptor, suggesting that it is very flexible. Z.Z. Wang proposed (Wang et al., J. Neurosci. 24:12625, 2009) that RIC3 pulls a7R subunits together by interactions between pairs of RIC3 coiled-coil domains and additional unidentified interactions between the RIC TM helix and a7R subunits. Selected alanine substitutions in CyM4AH (Castillo et al., J. Biol. Chem. 280:27062, 2005) blocks RIC3's ability to assemble a7Rs and these a7R wild-type amino acid side chains mostly project into the groove. Upon a7R assembly, the groove is blocked by overlap of CyM4AHs and by the loop between M3 and CAH from the neighboring subunit, requiring RIC3 to leave during the assembly process. **Method:** Chimeras made by replacing a7R TM domains with 5HT3 receptor helices assemble in HEK cells without any RIC3. We are looking for a7R amino acid substitutions that allow assembly (measured by alpha-bungarotoxin binding) while restoring RIC3 activity. **Preliminary results:** Replacing the chimeric 5HT3 M4 C-terminal with the SAP-latch sequence substantially blocks pentameric assembly. **Future directions:** Can RIC3 reverse the effects of the SAP-latch substitution if 5HT3 amino acid side chains that project into the groove are replaced with those of a7Rs?

Disclosures: R.H. Loring: None. **K. Agarwal:** None. **M. Gupta:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.08

Topic: B.02. Ligand-Gated Ion Channels

Support: Agencia Nacional de Promoción Científica y Tecnológica

Title: Study of the structural determinants involved in the potentiation of the a9a10 cholinergic nicotinic receptor by extracellular calcium.

Authors: *S. GALLINO[1], P. CRAIG[2], J. BOFFI[1], P. PLAZAS[3], B. ELGOYHEN[1,3];
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Abstract: The α9α10 nicotinic cholinergic receptor (nAChR) mediates the inhibitory synapse between medial olivocochlear efferent neurons and outer hair cells of the cochlea. The inhibition results from calcium (Ca^{2+}) entry through the α9α10 nAChR, and the subsequent activation of a small-conductance SK2 Ca^{2+}-dependent potassium channel. The α9α10 nAChR plays a key role in auditory neural circuits at the post-hearing onset. Hence, a deep understanding of the modulatory effects of this cholinergic receptor in auditory circuits would be extremely useful for the development of new translational strategies towards hearing loss and hyperacusis.The α9α10 nAChR is a pentameric cation-permeable ion channel that is composed of α9 and α10 subunits. Each nAChR subunit comprises a large extracellular amino-terminal domain, four transmembrane domains (TM1-TM4), a long cytoplasmic loop between TM3 and TM4 and a C-terminal domain. Expression of rat α9 and α10 nAChR subunits in *Xenopus laevis* oocytes yields functional α9 and α9α10 receptors, but not α10 homomeric nAChRs. One of the functional differences between α9 and α9α10 nAChRs is the modulation of their ACh-evoked responses by extracellular Ca^{2+}. While α9 nAChRs responses are blocked by Ca^{2+}, ACh-evoked currents through α9α10 nAChRs are potentiated by Ca^{2+} in the micromolar range and blocked at millimolar concentrations.In order to identify the structural determinants responsible for Ca^{2+} potentiation, we generated several chimeric and mutant α10 subunits, expressed them in *Xenopus* oocytes and performed electrophysiological recordings under two electrode voltage clamp. Our results suggest that the TM2-TM3 loop of the α10 subunit contains structural determinants responsible for the potentiation of the α9α10 nAChR by Ca^{2+}. Moreover, we identified α10 E 45 and E 175 as possible key residues of two potential Ca^{2+} binding sites involved in this potentiation.
These studies are being complemented with molecular dynamics simulations of the interaction of

Ca^{2+} with different nAChRs models (α9 and α10 homomeric receptors, and α9α10 heteromeric receptor in grouped or alternated subunit spatial arrangements) to help in the structural interpretation of the experimental results obtained.

Disclosures: S. Gallino: None. **P. Craig:** None. **J. Boffi:** None. **P. Plazas:** None. **B. Elgoyhen:** None.

Poster

P086. Nicotinic Acetylcholine Receptors: Structure, Function, and Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P086.09

Topic: B.04. Ion Channels

Support: NS-064969
GM-121463

Title: Efficiency: an agonist attribute that links binding and gating

Authors: *D. C. INDURTHI[1], A. AUERBACH[2];
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Abstract: Agonists are evaluated by a concentration-response curve (CRC), with a midpoint (EC_{50}) that indicates potency and a high-concentration asymptote (r_{max}) that indicates efficacy. The low-concentration asymptote indicates constitutive activity and is agonist-independent. A third agonist attribute, efficiency (η; eta) is the fraction of binding energy that is applied to the conformational change that activates the receptor. We show that η can be calculated from EC_{50} and the asymptotes of a CRC for muscle nicotinic acetylcholine receptors (AChR) derived from either single-channel or whole-cell responses. For 20 agonists the distribution of η values is multimodal with population means at 55% (including TMA), 51% (including ACh, nornicotine and DMPP), 42% (including epibatidine and cytisine) and possibly 35% (including varenicline). Agonist potency and efficacy are related by η, which otherwise appear to be unrelated. The value of η decreases with increasing volume of the agonist's head-group. Many binding site mutations have only a small effect on ACh efficiency except for αY190A (35%), αW149A (60%) and those at αG153 (42%, including one that causes CMS). If η is known, EC_{50} and r_{max} can be calculated from each other. Hence, an entire CRC can be estimated from the response to a single agonist concentration, and efficacy can be estimated from CRCs that have been normalized to 1. Given η, the level of constitutive activity can be estimated from a single CRC. Efficiency is the missing link between affinity and efficacy.

Disclosures: D.C. Indurthi: None. **A. Auerbach:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.01

Topic: B.09. Network interactions

Support: NIH R01 NS054281

Title: Stochastic population oscillations in type 2 networks of type inhibitory interneurons require neurons to be biased in a deeply subthreshold regime at low firing rates

Authors: *R. BARAVALLE, C. C. CANAVIER;
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Abstract: The leaky integrate and fire (LIF) model has been particularly useful in explaining the irregular firing of cortical neurons. An important candidate mechanism for the generation of gamma oscillations, the stochastic population oscillator is based on networks of LIF model neurons in the fluctuation driven regime. The network is amenable to a 1D Fokker Plank analyses assuming a fixed spike threshold and a random walk in the membrane potential plus a drift towards an effective subthreshold membrane potential. Pyramidal neurons in general have type 1 excitability, meaning they function as integrators and can fire arbitrarily slowly, so this is likely a good approximation. However, PV+ FS interneurons known to play an important role in gamma oscillations have type 2 excitability and cannot support repetitive firing below an abrupt cutoff frequency. Spiking in these neurons likely arises via a subcritical Hopf bifurcation. At subthreshold values of the membrane potential near the bifurcation, an unstable limit cycle surrounding the stable rest potential forms the spike “threshold”. This threshold may be crossed not only by depolarization, but also by hyperpolarization causing post-inhibitory rebound. Moreover, once the threshold is crossed the angular velocity of the vector field may allow multiple cycles (spikes) before the potential becomes “subthreshold”, enabling a phenomenon called stuttering. Therefore, it is not clear that the 1D Fokker Planck analyses that apply to type 1 neurons strictly apply to type 2 neurons nor whether the range of dynamic activity exhibited by networks of type 2 neurons is the same as type 1 neurons. Near the bifurcation a 2D Fokker Planck analysis may be required. The activity of networks of inhibitory LIF neurons has been characterized in the 2D parameter space of current noise intensity and synaptic conductance strength (Brunel and Hansel Neural Comput 18: 1066-1110, 2006) at a constant average firing rate, which eliminated bias current as a parameter. We have sampled the 3D parameter space including bias current for the Izhikevich cubic integrate and fire model with type 2 parameters. We show that type 2 neurons can exhibit a stochastic population oscillation, but only when sufficiently subthreshold that the vector field near the Hopf bifurcation or the saddle node of periodics is not sampled and with individual neuron firing rates much lower than the frequency of the oscillation. These results hold for nested gamma nested evoked by sinusoidal theta drive rendering this an unlikely mechanism for hippocampal theta nested gamma.

Disclosures: R. Baravalle: None. **C.C. Canavier:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.02

Topic:

Support: NIH R01NS054281

Title: Fast spiking interneurons generate gamma oscillations in the medial entorhinal cortex in the absence of excitatory cell input

Authors: *B. D. WILLIAMS[1], F. R. FERNANDEZ[1], C. C. CANAVIER[2], J. A. WHITE[1]; [1]Dept. of Biomed. Engin., Boston Univ., Boston, MA; [2]Dept. of Cell Biol. and Anat., Lousiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Many cells in layer II of the medial entorhinal cortex (mEC) exhibit spatially tuned firing rates that generate a grid-like ('grid cells') pattern when traversing an open field. Grid cell firing rates are modulated by a theta (4-12 Hz) frequency network-wide oscillation. Further, higher frequency gamma (40-140 Hz) oscillations are nested within the slower-wave theta oscillation and are believed to help synchronize grid cell spike output (Hafting et al., 2008; Reifenstein et al., 2012). Two different (but not mutually exclusive) mechanisms have been proposed to generate gamma oscillations. In the pyramidal-interneuron network gamma (PING) model, excitation of pyramidal cells activates local interneurons which provide inhibitory feedback to the pyramidal cells. Alternatively, the interneuron network gamma (ING) model proposes that inhibition between interneurons can lead to synchronous inhibition. To address the potential network mechanisms of theta-nested gamma, we used intracellular recordings in slices to measure excitatory and inhibitory post synaptic currents in stellate, pyramidal, and fast spiking cells during optogenetic stimulation. No differences were observed in the gamma frequency, theta phase or maximum power of the inhibition-driven gamma oscillations among the main cell types in the mEC. Excitation-driven gamma oscillations were observed in fast spiking interneurons, but not excitatory cells, consistent with the low degree of recurrent excitatory connections (Fuchs et al., 2016). Importantly, inhibition-driven gamma oscillations persisted after AMPA/kainate receptors were pharmacologically blocked, contrary to a similar study (Pastoll et al., 2013), but consistent with local field potential recordings in mEC (Butler et al., 2018). In support of the ING model, preliminary data indicates that inhibition-driven gamma oscillations can be observed when exclusively activating PV+ interneurons with optogenetic drive. These results suggest that a fast-firing inhibitory network is sufficient for generating gamma oscillations in the mEC. Therefore, both PING and ING mechanisms likely contribute to theta-nested gamma oscillations in the mEC. References: [1] Hafting et al. *Nature* (2008)

453:1248-52. [2] Reifenstein et al. *PNAS* (2012) 109:6301-6306. [3] Fuchs et al. *Neuron* (2016) 89:194-208. [4] Pastoll et al. *Neuron* (2013) 77:141-154. [5] Butler et al. *Eur J Neurosci.* (2018) 48:2795-2806.

Disclosures: B.D. Williams: None. **F.R. Fernandez:** None. **C.C. Canavier:** None. **J.A. White:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.03

Topic: B.09. Network interactions

Support: NIH R01 NS054281

Title: Simulated optogenetic theta stimulation is sufficient to evoke nested gamma in a biophysically realistic model of a fast spiking interneuronal network in the medial entorhinal cortex

Authors: G. VIA[1], F. R. FERNANDEZ[2], B. D. WILLIAMS[2], J. A. WHITE[2], *C. C. CANAVIER[1];
[1]Cell Biol. and Anat., LSU Hlth., New Orleans, LA; [2]Biomed. Engin., Boston Univ., Boston, MA

Abstract: Theta nested gamma oscillations in the hippocampal formation are thought to enable temporal codes with distinct items encoded by each gamma cycle. In vitro experiments have recently shown that exclusively activating PV+ interneurons with optogenetic drive at theta frequency drive is sufficient to evoke nested gamma in the medial entorhinal cortex (MEC) in the absence of excitation (see companion poster BD Williams et al.). In order to better understand how an interneuronal network gamma (ING) model can account for these results, we thoroughly characterized variability of MEC PV+ fast spiking interneurons and their connectivity. Variability in the electrophysiology was characterized in the input resistance, membrane time constant, resting potential, excitability and frequency/current (F/I) curves. We constructed a population of one compartment models with a leak conductance, a capacitance, a voltage-gated Na+ channel, and two voltage-gated K+ channels, Kv1 and Kv3. The passive properties (leak reversal, capacitance, leak conductance) were adjusted in a biophysical range. However, this was not sufficient to capture the full variability in the F/I curve. Variability in the conductance and half activations of the active currents was required to fit the population envelope of F/I curves in terms of slope, rheobase and the cutoff frequency below which these neurons cannot sustain firing. Imposing a linear correlation between the Kv3 and NaV peak conductance values and their half activation voltages helped constrain the models in a

physiological range. 100 model neurons were connected sparsely and randomly according to the experimental data with a probability of 0.6 via inhibitory synapses with delays uniformly distributed in (0.6,1.0) ms, a log-normal distribution of peak conductance, and rise and decay times of 0.4 and 2.3 ms, respectively. The short-term depression exhibited at PV+ to PV+ synapses (Fernandez et al. SFN Global Connectome poster P071.02, online, 2021) was incorporated into the network. In accordance with Pastoll et al 2013 (*Neuron* 77:141-154), during the peaks of theta the interneurons were above threshold in the mean driven regime. Despite the multiple dimensions of heterogeneity in the network, simulated sinusoidal activation of channel rhodopsin robustly evoked gamma oscillations, in accordance with our experimental observations. Moreover, as in Fernandez et al. 2021, a simulated stellate cell population spike during the rising phase of theta greatly increased gamma amplitude, suggesting that although ING is sufficient for theta-nested gamma oscillations, excitation synergistically amplifies the oscillation.

Disclosures: G. Via: None. **F.R. Fernandez:** None. **B.D. Williams:** None. **J.A. White:** None. **C.C. Canavier:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.04

Topic: B.09. Network interactions

Support: NIH R01 MH123687

Title: Frequency-, phase-, and task- specific functional connectivity across primate anterior cingulate and prefrontal cortex and striatum

Authors: *K. BANAIE BOROUJENI[1], T. WOMELSDORF[2];
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Abstract: Neural circuits in the fronto-striatal network are recurrently connected. This network structure suggests that interconnected neurons activate near-simultaneously with small time-lags between activations. Time-lags of activations should indicate the direction of spike-transfer and hence neural communication, but so far studies in primates quantified time-lags of spiking activation mostly indirectly using the local field potential. To surmount this limitation, we developed and applied a novel technique that delineates the time-lagged functional connectivity based solely on spiking activity across network. We, first, validated with biophysically plausible network modelling a novel technique to characterize time-lagged connectivity based on single neuron spike-triggered modulation of multiunit activity. We then applied the technique to simultaneously recorded spiking activity from the lateral prefrontal cortex (LPFC), anterior

cingulate cortex (ACC), and anterior striatum in two rhesus monkeys while they were engaged in an adaptive learning task. We found that spike-triggered functional connectivity profiles overall match anatomically expected connectivity profiles. The strength and sign of functional connectivity was significantly modulated by the frequency and the phase of transient oscillatory events. Spikes in ACC and LPFC occurring during transient oscillatory bursts had stronger impact on each other and the striatal circuit. This oscillatory gain on spike-efficacy was in the beta band in LPFC to the striatum, and in the theta band in ACC to the LPFC. These findings are likely functionally important as we found stronger functional connectivity for neuron-multiunit pairs that were tuned to the reward outcomes during learning. These results elucidate frequency-, phase-, and task- specific modulation of spiking co-variation, and thus depict principles of neural communication in the fronto-striatal network during adaptive learning behavior.

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

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.05

Topic: B.09. Network interactions

Support: MH122023

Title: Parsing mechanisms that support theta rhythm in a hippocampal CA3 model

Authors: *P. CANFIELD[1], M. STROUD[1], V. GUNTU[1], A. M. HUMMOS[2], S. S. NAIR[1]; [1]Electrical & Computer Engin., [2]Informatics Inst., Univ. of Missouri, Columbia, MO

Abstract: We report a computational model of the CA3 region of a rodent hippocampus to characterize the relative contributions of the intrinsic physiological mechanisms to the generation of the intrinsic CA3 theta rhythm. In the network model, the CA3 module receives non-rhythmic excitation from the entorhinal cortex (EC) and from the dentate gyrus (DG) modules. The DG module also receives input from EC as in our previous model (Hummos et al., 2017). Specifically, we extend our previous studies and parse the intrinsic physiological theta-generating mechanisms into resonant and synchronizing types and show that such a categorization can help predict the mechanisms that would interfere or possibly substitute for another, in the generation of the theta rhythm. A mechanism is termed resonant if it produces rhythms via the intrinsic dynamics of components participating in the mechanism, e.g., slow inhibition, spike-frequency adaptation, slow neuronal currents, and rhythmic external input. And it is termed synchronizing if it support rhythm coordination, e.g., non-rhythmic external excitation, inhibitory feedback, and recurrent excitatory connections. Some of the intrinsic mechanisms were found to participate in both functions. We found that pyramidal cell adaptation

interferes with theta rhythms produced by slow inhibition and that fast inhibition can substitute for rhythm generation or interfere with it through slow inhibition, depending on the level of neuromodulation (via acetylcholine). These results could help reconcile conflicting reports about the effects on rhythm generation by the inactivation of specific circuit components. We find that the effects of component removal can only be characterized in the context of the mechanisms present and on the neuromodulatory state of the system.

Disclosures: P. Canfield: None. **M. Stroud:** None. **V. Guntu:** None. **A.M. Hummos:** None. **S.S. Nair:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.06

Topic: B.09. Network interactions

Support: MH122023

Title: Linking Oscillating Cell Assemblies and Population Vectors in a Model Neocortical Circuit

Authors: *Z. CHEN[1], D. DOPP[3], D. B. HEADLEY[4], S. S. NAIR[2];
[2]Electrical & Computer Engin., [1]Univ. of Missouri Columbia, Columbia, MO; [3]Univ. of Missouri, Columbia, MO; [4]Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Rhythmic activity is closely linked to neuronal processing within and across brain regions. The microcircuit mechanisms that subserve such rhythms via intrinsic and extrinsic inputs are being studied actively. Neocortical circuits exhibit both beta and gamma rhythms which are through to be caused by distinct subcircuits involving different type of interneurons. Another approach to study the microcircuit mechanisms is via population vectors. However, it is not clear how rhythmically oscillating cell assemblies and population vectors are related, and how they influence each other.
We report a 1000-cell biophysical computational model of a neocortical circuit to investigate how different patterns of population activity recruit specific beta and gamma generating micro-circuits. Our preliminary results with a 1000-cell network model (Feng et al., 2020) indicated that stable afferent ensembles recruit beta while varying ensembles recruit gamma oscillations. Previous work on gamma and beta oscillations have emphasized the resonant properties of interneuron networks as being instrumental in rhythm generation. Our preliminary model indicated that this is only part of the reason for the emergence of brain rhythms. The frequency of the rhythm is largely determined by the resonance of the recurrent principal cells/interneuron network. However, whether that network is activated, and the persistence of that activity,

depends upon the slower temporal dynamics of the principal cell ensembles that supply the excitation to fuel the rhythm. Our preliminary model did not have specific layers and had only one type of pyramidal cells. The revised network model includes all pertinent layers in the neocortex with their predominant cell types. Model single cell characteristics as well as network features such as cell numbers, connectivity, synaptic and short- and long-term plasticity characteristics were calibrated using biological reports.
One direction that on-going work explores is how random extrinsic drive engages the population vectors. This is performed by varying either the drive intensity or the ensemble recruited. Another direction explores activation of different ensembles and how the population vector trajectory on the intrinsic manifold transitions between different ensembles.

Disclosures: Z. Chen: None. **D. Dopp:** None. **D.B. Headley:** None. **S.S. Nair:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.07

Topic: B.09. Network interactions

Support: NIH-MH122023 (SSN)

Title: Generation of oscillatory LFP bursts in different model brain regions

Authors: *M. STROUD[1], Z. CHEN[2], S. S. NAIR[3];
[1]Univ. of Missouri, Columbia, Harrisburg, MO; [2]Electrical Engin. and Computer Sci., [3]Electrical & Computer Engin., Univ. of Missouri Columbia, Columbia, MO

Abstract: Generation of oscillatory LFP bursts in different model brain regions Matthew Stroud, Ziao Chen, Feng Feng, Satish S. NairElectrical Engineering & Computer Science Dept., University of Missouri, Columbia, MO
Abstract A recent physiologically constrained biophysical network model of the rodent amygdala produced gamma (30-80 Hz) bursts as transient, several cycle-long bursts, via the PING mechanism involving principal cells (PNs) and fast-spiking interneurons (ITNs). Moreover, the model local field potential (LFP) mimicked the fine structure of gamma rhythmogenesis seen *in vivo* (Feng et al., 2019). We developed a similar 1000-cell model of a structurally different rodent region, the layered neocortex, to explore the dynamics that engender two prevalent types of bursts seen in the LFP from neocortex: beta, and gamma. The present study compares the dynamics related to burst generation between the nuclear-structured amygdala with randomly projecting axons, and the layer-structured neocortex with axons aligned in the same direction.
Specifically, this comparative study investigates how the interactions between the stochastic

extrinsic afferents and the intrinsic dynamics, including short-term synaptic plasticity, impact both the generation and the characteristics of bursts. We will also explore the formation of cell assemblies underlying the bursts as well as the capabilities and limitations of reduced order models and approaches such as mean-field models and population-averaging, in predicting the genesis and characteristics of bursts. Preliminary amygdala model results of average populational activity rates of afferents, PNs and ITNs after aligning by cycle phase showed cross-correlations with the gamma-band filtered LFP along expected lines but with a larger time lag with afferents: approximately one gamma cycle (~17 ms) for afferents, half gamma cycle (~8 ms) for PNs and a quarter of gamma cycle (~5 ms) for ITNs. These early results provided insights into both the temporal lags in correlations and possible interaction dynamics, which are being analyzed presently. A next step is to also compare these characteristics with those seen in the neocortical model to explore how a layered architecture might affect the generation of similar bursts.
In that model, the afferents were modeled as two separate components. One set comprised distinct uncorrelated Poisson spike trains, with only one impinging a cell via an excitatory synapse with synaptic weight drawn from a log normal distribution. The other set was modeled as uncorrelated point-conductances on the soma, with both excitatory and inhibitory components that had log-normally distributed weights.

Disclosures: M. Stroud: None. **Z. Chen:** None. **S.S. Nair:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.08

Topic: B.09. Network interactions

Support: NSERC Alexander Graham Bell Canada Graduate Scholarship – Doctoral (CGS D) award

Title: Cholecystokinin basket cell (CCK+BC) and parvalbumin basket cell (PV+BC) interactions are crucial elements for theta rhythm generation in a novel biophysical model of the rodent hippocampus.

Authors: *A. CHATZIKALYMNIOU[1,2], F. K. SKINNER[1,2];
[1]Krembil Res. Inst., Toronto, ON, Canada; [2]Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: In the hippocampus, the 3-12 Hz local field potential (LFP) theta oscillation is strongly correlated with spatial navigation, episodic memory, and rapid-eye-movement (REM) sleep. Understanding the origin of theta rhythms could shed light on the encoding and retrieval of cognitive information. However, how the large variety of cell types form a theta-generating network remains unknown. We use a novel CA1 hippocampus model of unprecedented

biological detail to uncover the generation mechanism of theta rhythms. The model contains detailed representations of eight inhibitory cell types and pyramidal cells and is constructed based on experimentally derived connectivity. We find that a 400 μm thick, isolated segment (slice) represents the minimum network that can produce these activities. Guided explorations of this computationally tractable model reveals that theta rhythms are initiated by the pyramidal cells whose net input drive is strongly correlated with the resulting theta frequency. This implies that the intrinsic properties of the pyramidal cells are theta frequency controllers. Returning to the full hippocampus model, we study the role of inhibition to the generation of theta rhythms. Of the eight inhibitory cell types in the model, abolishment of three types, the PV+BCs, bistratified cells (BiCs) and CCK+BCs, is particularly disruptive to theta. With respect to PV+BCs and BiCs, we find that these cell types reset the theta rhythm by mediating the sharp termination of pyramidal cell theta bursts. The CCK+BCs on the other hand are found to be prominent controllers of the PV+BC excitability. Specifically, a comprehensive grid search of the nine postsynaptic weights from the different cell types onto the PV+BCs shows that small changes to the CCK+BC-PV+BC postsynaptic weights can induce drastic alterations to the PV+BCs' firing rates. Consequently, theta rhythmicity relies on balanced activation of both CCK+BCs and PV+BCs which, if disrupted, drives the network out of the theta oscillatory regime. In conclusion, our models can be used to identify cellular-specific pathways underlying theta and theta-gamma modulations in behaving animals. In turn, this opens up avenues for clinical translation where theta (and gamma) rhythms are pathologically modulated.

Disclosures: A. Chatzikalymniou: None. **F.K. Skinner:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.09

Topic: B.09. Network interactions

Support: ANR-18-CE37-0014-02

Title: Stimulation response is shaped by the brain's collective dynamical state

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Abstract: Neuronal populations within local regions frequently undergo oscillatory modulations of their activity. The oscillations of distant populations coupled by long-range connections can phase lock at different stable phase differences. The flexible change of these phase locking

patterns between brain regions has been hypothesized to modulate communication and functional interactions between them. A tool which has proved useful to model and predict the behavior of coupled oscillating populations is the phase response curve (PRC). The PRC is a local transformation function that determines the phase-dependent response of an oscillator to any given input. More precisely, the PRC quantifies the phase advancement or delay of the oscillation in a stimulated region, changing as a function of the phase at which a brief stimulation pulse is applied. Such phase shifts can persist after stimulation and may even lead to a modified functional connectivity of the stimulated region with other regions. However, the notion of PRC has been formulated for isolated oscillating systems. On the contrary, brain regions are part of a broader system, interacting with many other regions via a structural connectome. The oscillatory dynamics of large-scale brain networks is collective in nature and the behavior of individual regions composing them can strongly differ from the behavior of these regions in isolation. The phase-shifting effects produced by a local pulse perturbation may thus not depend uniquely on the phase of the ongoing local oscillation, as assumed by the PRC framework, but also on the state of the ongoing whole-brain dynamics. If this was the case, predictions of the phase-shifting effects induced by stimulation based on conventional PRCs may radically deviate from reality. Here, we construct computational models of whole-brain dynamics and tune them to produce complex dynamics with switching between spontaneously occurring functional connectivity states. These functional connectivity states are characterized by distinct cores of strongly integrated regions and different sets of stable phase lags between oscillating regions. We then measure the "effective PRC" of selected brain regions in different functional connectivity states by quantify the phase-shifting effects of the stimulation in a phase-dependent manner in each state. We find that these effective PRCs not only differ strongly from the conventional PRCs in the uncoupled regions, but also differ across functional connectivity states. Our results suggest that it is crucial to consider the collective dynamical state of a network, such as the brain, when predicting the effect of stimulation.

Disclosures: S. Benitez Stulz: None. **G. Rabuffo:** None. **G. Dumont:** None. **B.S. Gutkin:** None. **D. Battaglia:** None.

Poster

P132. Oscillations and Synchrony: Computer Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P132.10

Topic: B.09. Network interactions

Support: "Institut de Convergence ILCB" (ANR-16-CONV-0002)

Title: Low-level algorithmic decomposition of neural circuit functions

Authors: N. VOGES[1], A. BROVELLI[1], ***D. BATTAGLIA**[2];
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Abstract: Cognitive functions must arise from the coordinated activity of neural populations distributed over large-scale brain networks. However, it is challenging to understand how specific aspects of neural dynamics translate into operations of information processing, and, ultimately, cognitive function. An obstacle is that simple circuit behaviors -such as self-sustained or propagating activity and nonlinear summation of inputs- do not directly give rise to high-level functions, even if they do, nevertheless, implement simple transformations of the information carried by neural activity. In other words, they already map to low-level processing operations, that can be used within more complex functional computations.
Here we show that distinct neural circuit functions, such as stimulus representation, working memory or selective attention stem from different combinations and types of low-level processing operations. To prove this hypothesis, we combine approaches from information theory with computational simulations of canonical neural circuits involving one or more interacting brain regions and emulating well-defined cognitive functions.
More specifically we track the dynamics of information emergent from neural activity dynamics, using suitable quantitative metrics to detect where and when information is actively buffered ("active information storage"), transferred ("information transfer") or non-linearly merged ("information modification"), as different possible modes of low-level processing. We thus find that neuronal subsets maintaining representations in working memory or performing attention-related gain modulation are signaled by their boosted involvement in operations of, respectively, active information storage or synergistic modification.
Thus, information dynamics metrics, beyond detecting *which* network units participate in cognitive processing, also promise to specify *how* they do it, i.e. through which type of primitive computation, a capability that could be exploited for the parsing of actual experimental recordings.

Disclosures: N. Voges: None. **A. Brovelli:** None. **D. Battaglia:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.01

Topic: B.11. Glial Mechanisms

Support: NIH R00 AG056662

Title: Sexually dimorphic effects of astrocyte-specific superoxide dismutase deficiency on cognitive function

Authors: *S. LOGAN[1,2], R. Y. NAGARAJA[3], M. BAIER[4], H. YARBROUGH[4], A. MASINGALE[1], M.-P. AGBAGA[3];
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Abstract: Cognitive decline is a debilitating aspect of aging and neurodegenerative diseases such as Alzheimer's disease that is closely associated with mitochondrial dysfunction, increased reactive oxygen species (ROS) and accumulation of damaged macromolecules that are hallmarks of cellular aging. The purpose of this study was to investigate the effects of increased mitochondrial oxidative stress specifically in astrocytes, using tamoxifen-inducible astrocyte-specific knockout of SOD2 (*aSOD2-KO),* on neuronal function and cognitive performance. We reduced astrocyte levels of mitochondrial superoxide dismutase (SOD2) to induce mitochondrial oxidative stress in both males and females at 5-6 months of age and learning and memory was assessed using the PhenoTyper and radial arm water maze at 15 months of age. We show that aSOD2-KO impairs hippocampal-dependent spatial working memory. Male aSOD2-KO mice showed decreased cognitive flexibility in the Reversal phase in the PhenoTyper and the radial arm water maze. No significant differences were observed in the females. aSOD2-KO males further showed decreased hippocampal long-term potentiation, while paired-pulse facilitation was unaffected. Levels of D-serine were also reduced in aSOD2-KO males and in primary cultured astrocytes treated with MitoPQ. Furthermore, when challenged with NMDA toxicity, aSOD2-KO males demonstrated increased neuronal death in the hippocampus and hypothalamus compared to age-matched controls. These results suggest that post-synaptic mechanisms may underlie the associated cognitive dysfunction with increased oxidative stress in astrocytes possible through reduced D-serine availability and impaired glutamate handling. Thus, targeting astrocytic mitochondrial function may be an avenue for therapy for age-related cognitive impairment.

Disclosures: S. Logan: None. **R.Y. Nagaraja:** None. **M. Baier:** None. **H. Yarbrough:** None. **A. Masingale:** None. **M. Agbaga:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.02

Topic: B.11. Glial Mechanisms

Support: NRF-2019R1A2C1084232
KBRI-21-BR-01-05

Title: Hyperactive glial BRAF-ERK signaling impairs learning and memory in adult mice

Authors: M. KANG[1], J. HAN[2], J. CHOI[3], H.-H. RYU[1], P. PARK[4], S. KIM[1], J. CHOI[4], D. HAN[4], B.-K. KAANG[4], B. G. NEEL[5], C. KIM[3], H. PARK[2], *Y.-S. LEE[1];
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Abstract: Germline mutations in RAS signaling pathway are associated with various developmental disorders such as Noonan syndrome, neurofibromatosis, Cardio-facio-cutaneous syndrome (CFCS) and so on, collectively called RASopathy. Mutations in *BRAF* gene encoding a downstream effector of RAS account for majority of CFCS. Most of CFCS patients display severe degrees of cognitive impairments such as intellectual disability, but the neurobiological basis of their cognitive abnormalities remains largely unknown. Here, we investigated how the aberrant BRAF signaling affects cognitive functions using conditional knock-in mice harboring CFCS-associated *BRAF* mutations. Expressing the mutant BRAF under control of Nestin-, but not αCaMKII- or vGAT-CRE, resulted in severe hippocampal memory deficits in mice. Intriguingly, we found that the mutant *BRAF* increased the number of reactive-like astrocytes in multiple experimental systems including mutant mice, cultured mouse astrocyte, and human cortical organoid, suggesting that the mutant BRAF and subsequently activated ERK signaling renders astrocytes being in reactive-like status. Importantly, astrocyte-specific expression of the mutant BRAF in adult was sufficient to induce cellular and behavioral deficits in mice. Our study demonstrates that the reactive-like astrogliosis may underlies the severe cognitive deficits in CFCS.

Disclosures: M. Kang: None. **J. Han:** None. **J. Choi:** None. **H. Ryu:** None. **P. Park:** None. **S. Kim:** None. **J. Choi:** None. **D. Han:** None. **B. Kaang:** None. **B.G. Neel:** None. **C. Kim:** None. **H. Park:** None. **Y. Lee:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.03

Topic: B.11. Glial Mechanisms

Support: 1R01NS118569
R00AG048222
Alzheimer's Association
Leon Levy Foundation
Swid Family

Title: Dementia-linked TDP-43 dysregulation in astrocytes impairs memory, antiviral signaling, and chemokine-mediated astrocytic-neuronal interactions

Authors: *A. LICHT MURAVA[1], S. M. MEADOWS[2], F. PALAGUACHI[1], S. C. SONG[4], Y. BRAM[5], C. ZHOU[3], R. E. SCHWARTZ[5], R. C. FROEMKE[4], A. L. ORR[1], A. G. ORR[2]; [1]Brain and Mind Res. Inst., [2]Brain and Mind Res. Institute, Weill Cornell Grad. Sch., [3]Brain and Mind Res. Institute, Tri-Institutional MD-PhD Program, Weill Cornell Med., New York, NY; [4]New York Univ. Sch. of Medicine, Skirball Inst., New York, NY; [5]Dept. of Med., Div. of Gastroenterology and Hepatology, Weill Cornell Med., New York, NY

Abstract: Dysfunction in transactive response TAR DNA-binding protein 43 (TDP-43) is linked to cognitive deficits in diverse neurodegenerative disorders, including frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD). The effects of TDP-43 pathology in different cell types, including astrocytes, are not clear. In this study, we investigated whether astrocytic TDP-43 is dysregulated in human brain and how this dysregulation affects behavior, gene expression, and neural cell functions. Our results show that astrocytic TDP-43 is mislocalized in postmortem human hippocampal tissue from AD cases. To assess the effects of widespread or hippocampus-specific dysregulation of astrocytic TDP-43 in complementary systems, we generated three novel astrocyte-specific mouse models of TDP-43 dysfunction. Consistently, these mouse models indicated that astrocytic TDP-43 dysfunction causes progressive hippocampus-dependent memory loss, but not motor deficits. Manipulation of astrocytic TDP-43 also increased hippocampal levels of interferon (IFN)-inducible chemokines CXCL9 and CXCL10, and altered cell-autonomous antiviral signaling and defense against viral pathogens. Moreover, expression of CXCR3, the shared receptor for CXCL9 and CXCL10, was increased selectively in hippocampal presynaptic terminals. Acute or chronic stimulation of presynaptic CXCR3 modulated neuronal activities and presynaptic vesicles. Overall, our findings shed new light on TDP-43 dysregulation in astrocytes and its potential contributions to disease-related impairments in cognitive and immune-related functions. We report a novel chemokine-mediated astrocytic-neuronal pathway that is likely downstream of aberrant antiviral immune signaling in astrocytes that affects presynaptic release and neuronal activities. Together, these results implicate astrocytic TDP-43 dysregulation in the pathogenesis of dementia and point to chemokine signaling and CXCR3 as potential therapeutic targets for alleviating cognitive decline.

Disclosures: A. Licht Murava: None. **S.M. Meadows:** None. **F. Palaguachi:** None. **S.C. Song:** None. **Y. Bram:** None. **C. Zhou:** None. **R.E. Schwartz:** None. **R.C. Froemke:** None. **A.L. Orr:** None. **A.G. Orr:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.04

Topic: B.11. Glial Mechanisms

Title: Bmp4 regulates lipid metabolism and unfolded protein response in astrocytes

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Abstract: Alzheimer's disease (AD) is the most prevalent and debilitating conditions affecting elderly populations. Aging and the *ε4* allele of Apolipoprotien E (ApoE) are known as the greatest risk factors of the disease, yet molecular mechanisms connecting the two factors are unknown. Previous research has indicated that the levels of Bone Morphogenetic Protein 4 (BMP4) increase dramatically as individuals age, and we hypothesize that such increases in BMP4 expression play a role in APOE mediated lipid metabolism and cellular stress response correlated with AD pathogenesis in astrocytes, which produce the majority of APOE in the brain. To determine the effects of BMP signaling on endoplasmic reticulum (ER) stress associated unfolded protein response (UPR) and lipid droplet (LD) formation in astrocytes, primary murine astrocytes were treated with BMP4, Noggin (a BMP antagonist), GSK26066414 (a UPR pathway inhibitor) in the presence or absence of Brefeldin A or Tunicamycin-induced ER stress for 24 hours. Following treatment, the levels of GRP78, a UPR marker, and APOE were evaluated by western blot, and the levels of APOE, GRP78, and lipid droplet formation were evaluated by immunocytochemical staining. The results indicated that, not only can BMP4 induce UPR in astrocytes, which is accompanied by an increase in the formation of LD, but the induction of the astrocytic UPR is dependent on BMP signaling as Noggin treatment reduce UPR and LD formation under ER stress conditions. In addition, upregulation of APOE by BMP signaling activation takes place independent of the UPR activation. We will also present findings from parallel experiments using APOE deficient astrocytes to determine whether APOE is necessary for BMP mediated UPR and LD phenotypes. Together, these studies will elucidate the role of BMP signaling in the context of astrocytic responses to the AD pathological cascades.

Disclosures: S.A. Kessler: A. Employment/Salary (full or part-time):; Northwestern University. **A.K. Linden:** None. **T.L. McGuire:** A. Employment/Salary (full or part-time):; Northwestern University. **C. Peng:** A. Employment/Salary (full or part-time):; Northwestern University.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.05

Topic: B.11. Glial Mechanisms

Support: R01AA016959

Title: Three dimensional analysis of astrocyte phenotype in a rat model of alcohol use disorder

Authors: *S. P. GUERIN[1], K. NIXON[2];
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Abstract: Individuals with Alcohol Use Disorder (AUD) commonly experience cognitive impairments which result from structural and functional changes in the brain. Heavy alcohol consumption can cause both neuronal cell death and changes to glial phenotype with some data suggesting that females exhibit higher expression of markers typically upregulated in reactive astrocytes. In a rat model of AUD, increases in the expression of reactive astrocyte marker vimentin were observed in multiple brain regions, most dramatically in the hippocampus, after 7 days of abstinence from alcohol dependence in male rats. However, little is known about the effects in female rats and the expression of other markers commonly upregulated in reactive astrocytes have yet to be defined in this model. Therefore, adult female and male rats (PND 65-70; ~240g males, ~350g males) were subjected to a four-day binge ethanol paradigm of alcohol dependence. Ethanol (25% w/v in Vanilla Ensure Plus) was administered to animals 3 times per day for 4 days, while control animals were administered an isocaloric diet. Doses of ethanol were adjusted based on an intoxication behavior scale which resulted in an average dose of 9.5 ± 1.0 g/kg/day for males, and 10.1 ± 0.6 g/kg/day for females. Blood ethanol concentrations of 360.8 ± 63.1 mg/dl for males, and 344.9 ± 33.6 mg/dl for females were recorded. Withdrawal behaviors were observed starting 10 hours after the last dose for 17 hours. Model parameters were statistically similar between males and females. 7 days following the final dose, animals were transcardially perfused, brains were removed and postfixed, and sectioned in 12 series of 40µm sections. Fluorescent immunohistochemistry for GFAP, an intermediate filament protein that labels the proximal portion of astrocyte processes, and S100B, a calcium binding protein, were used to identify populations of astrocytes. Z-stack images of labeled cells in the hilus of the hippocampus were taken using a laser scanning confocal microscope. Imaris software was utilized to create three dimensional models of individual GFAP and S100B volumes, surface areas, and to reconstruct visible astrocyte processes. There were no significant differences between male ethanol and control groups in either S100B or GFAP immunoreactivity. Two-way ANOVA revealed a main effect of Sex in multiple measures of GFAP morphology such as GFAP sphericity and volume. These results suggest that the phenotype of reactive astrocytes following ethanol dependence is unique compared to other pathologies and may exhibit sex differences consistent with reports on female susceptibility to alcohol's damaging effects.

Disclosures: S.P. Guerin: None. **K. Nixon:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.06

Topic: B.11. Glial Mechanisms

Support: CureSMA (Audrey Lewis Young Investigator Award)
R21 NS102911

Title: The role of astrocytes in spinal muscular atrophy motor neuron synaptic defects

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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by the loss of lower spinal motor neurons. Evidence suggests that survival motor neuron (SMN) protein deficiency in other cell types can contribute to SMA pathology. We and others have demonstrated astrocytes derived from SMA patient induced pluripotent stem cells (iPSCs) show SMN-dependent intrinsic defects, including increased production and secretion of microRNA-146a which contributes to motor neuron cell loss. Our recent work shows elevated levels of microRNA-146a are still detected in SMA patient cerebral spinal fluid post-nusinersen treatment and may negatively impact iPSC-derived motor neuron functionality *in vitro*. Motor neuron peripheral and central synaptic defects have been described in SMA, but it remains to be fully determined how human glial cells contribute to synapse pathogenesis. Since astrocytes are known to play an important regulatory role in synapse development and function, we are investigating novel disease mechanisms involving astrocyte-mediated motor neuron synaptic defects in SMA. Differential gene expression analysis of our iPSC-derived astrocyte RNA seq data demonstrates a significant down-regulation of genes associated with synaptic transmission and plasma membrane cell projections in SMA patient iPSC-derived samples. Using the SurfaceGenie prediction tool, we verified many of these down-regulated genes are likely to encode cell surface proteins associated with ion gradient regulation, neurotransmitter uptake, and synaptic formation and integrity. We therefore hypothesize that SMA astrocytes may lack important synaptic-related cell surface genes and their encoding proteins, which could contribute towards motor neuron synaptic defects. Using cell surface capture mass spectrometry, we have identified cell surface proteins present in healthy and SMA patient-derived astrocyte samples. We are currently validating and quantifying protein candidates of interest using a targeted mass spectrometry approach. We will assess if candidate proteins are implicated in synaptic defects by applying super resolution microscopy and multi-electrode array (MEA) techniques to our iPSC-derived motor neuron astrocyte co-cultures. Thus far, initial MEA recordings reveal reduced motor neuron weighted mean firing rate and bursting activity when co-cultured with SMA patient-derived astrocytes. Identification of novel astrocyte cell surface proteins implicated in motor neuron synaptic defects could be targeted in future therapies for SMA.

Disclosures: E. Welby: None. **L. Berg Luecke:** None. **R.L. Gundry:** None. **A.D. Ebert:** None.

Poster

P159. Astrocytes: Disease Mechanisms II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P159.07

Topic: B.11. Glial Mechanisms

Support: NIH/NIA U19AG068054
NIH/NICHD HDR01064993
NIH/MARC T34GM136498
NIH/NIA P50AG16573
NIH/NIA P30AG066519
NIH/NIA R01AG21912
NIH/NIA U01AG051412

Title: Morphological and Distribution changes of Astrocytes in Alzheimer's Disease and Down Syndrome

Authors: *L. VAZQUEZ[1], A. C. MARTINI[1], M. J. PHELAN[1], E. HEAD[1,2,3,4];
[1]Dept. of Pathology and Lab. Med., [2]Inst. for Memory Impairments and Neurolog. Disorders, UCI, Irvine, CA; [3]Dept. of Neurol., [4]Dept. of Pharmacol. & Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Down syndrome (DS) or Trisomy 21 is the most common genetic cause of intellectual disability. People with DS express excessive amounts of the amyloid precursor protein (APP) gene on the triplicated chromosome 21, leading to an enhanced production of the beta-amyloid found in Alzheimer's Disease (AD). The majority of people with DS develop sufficient AD neuropathology by the age of 40; however, clinical symptoms of AD arise approximately a decade later. Neuroinflammation is a factor that has proven to be critically involved in AD development, and it holds a distinct pattern in people with Down syndrome and AD pathology (DSAD) compared to AD. Astrocytes are the most abundant glial cells in the brain, yet much is still unknown regarding their role and morphological differences in DSAD. Although astrocytes were categorized into two main subtypes known as A1 (neurotoxic) and A2 (neuroprotective), recent studies found these cells to be extremely heterogeneous and diverse, not adopting a single binary classification but rather a much more complex phenotype. These states may vary at different ages, cerebral areas, and neuropathological conditions. The goal of the present study is to characterize the varying distributions, morphologies, and states of astrocytes in the frontal cortex and posterior cingulate cortex of people with DS, DSAD and AD along the lifespan. With a combination of immunostaining markers, and morphological as well as quantification analysis using ImageJ, the heterogeneity of astrocytes will be assessed. These results will provide valuable insight into the roles of astrocytes in the development of AD in people with DS.

Disclosures: L. Vazquez: None. **A.C. Martini:** None. **M.J. Phelan:** None. **E. Head:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.01

Topic:

Support: NIMH MH10748
NIMH MH121102

Title: Protein Kinase A Expression and Activity in Alzheimer's Disease

Authors: *P. R. KANDIMALLA, S. SAHAY, N. D. HENKEL, R. E. MCCULLUMSMITH; Univ. of Toledo, Toledo, OH

Abstract: Since neurocognitive disorders are estimated to impact one in every ten American seniors, Alzheimer's disease (AD) has and will continue to have a prominent presence in our communities. Given that more than 140 clinical trials have failed to alter the progression or cure AD, a deeper understanding of the pathophysiology of this neurocognitive disorder is needed. Protein Kinase A (PKA) is a class of enzymes with a wide range of cellular homeostatic functions and is primarily involved in the cAMP-PKA signaling pathway. Past research has shown that the cAMP-PKA signaling pathway is dysregulated in AD. Using the PamGene12 Kinome Array on postmortem DLPFC brain, we identified PKA as an overrepresented "hit" on the array compared to age-matched, control (CTL) samples. We hypothesize that PKA expression (at the transcript, protein, and enzymatic activity levels) will be significantly higher in AD than that of mild cognitive impairment (MCI) and CTL samples. We will quantify changes in the expression of the PKA catalytic subunits (PRKACA and PRKACB) and its regulatory subunits (PRKAR1A, PRKAR2B, PRKAR2A, PRKAR1B, and PKIA) in the DLPFC and the hippocampus of our subjects (Male/Female CTL, n = 22/sex; Male and Female AD, n = 22/sex; Male and Female MCI, n = 22/sex). We will also quantify PKA protein levels using Western blotting protocols. Lastly, we will measure PKA activity in the same AD and control samples. This will be performed with an H89 inhibitor to normalize our quantifications. We expect our findings to support our hypothesis that PKA expression and activity is perturbed in AD compared to MCI and CTL samples.

Disclosures: P.R. Kandimalla: None. **S. Sahay:** None. **N.D. Henkel:** None. **R.E. McCullumsmith:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.02

Topic: H.08. Learning and Memory

Title: Assessment of the Active Kinome in Hippocampal Subfields

Authors: *E. S. SHEDROFF, N. D. HENKEL, P. PULVENDER, A. S. IMAMI, A. W. JOYCE, W. G. RYAN, V, K. ALGANEM, R. E. MCCULLUMSMITH;
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Abstract: This study will use a functional proteomic approach to examine the active kinome in the hippocampus. The active kinome is the global activity of all protein kinases in a system. The hippocampus is subject to dynamic change based on regulatory needs within the system and can therefore contribute to neuroplasticity at a cellular level. The hippocampus has distinct subfields (DG, CA1, CA2, CA3, CA4) and plays a major role in learning and memory. The hippocampal subfields are discrete and have distinct anatomy. Thus, we hypothesize that each subfield has a unique active kinome profile. Differential protein kinase regulation may be essential for molecular plastic changes within the brain. This plasticity is vital for cognitive ability.
By identifying the protein kinase activity in each hippocampal subfield, comparisons can be made between subkinomes to better understand signaling pathways associated with memory and learning within the brain. The subfields will be isolated via laser capture microdissection (LCM) and run on the PamGene kinome array. Analysis of the array data will be performed using bioinformatic pipelines. Bioinformatic analyses will identify the kinase activity profiles enriched in different hippocampal subfields . The pipelines used in kinomic analysis are Upstream Kinase Analysis (UKA) and Kinome Random Sampling Analyzer (KRSA). Additionally, pathways will be visualized using Bayesian Network Modeling (KINNET). Hippocampal subfield specific pathways will also be generated. The key kinases investigated and the pathways they belong to will then be compared and contrasted between subfields to paint a clearer picture of how they communicate with one another. These studies will extend our knowledge of signalling mechanisms in the hippocampus and provide a roadmap for interrogating the hippocampus in discrete subfields.

Disclosures: E.S. Shedroff: None. **N.D. Henkel:** None. **P. Pulvender:** None. **A.S. Imami:** None. **A.W. Joyce:** None. **W.G. Ryan:** None. **K. Alganem:** None. **R.E. McCullumsmith:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.03

Topic:

Support: U54 AG054345
NIH T32GM842528

Title: Connectomic analysis of whole brain network via glucose metabolism in the 5xFAD mouse

Authors: *R. M. SILVOLA[1], M. VERONESE[4], S. PERSOHN[2], A. A. BEDWELL[2], K. ELDRIDGE[2], R. SPEEDY[2], P. R. TERRITO[3];
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia worldwide, yet no effective strategies to prevent, slow, or reverse the disease exist. The number of people in the US affected by AD is expected to reach 13.8 million by 2050 and incur a financial burden of $9.12 trillion over this timeframe, thus highlighting a critical need for the development of novel diagnostic methods to better characterize onset and progression of disease. To address this gap, we adapted a novel connectomic analysis of mouse brain circuits and studied changes in glucose metabolism across time (ages), sexes, and genotypes in the 5xFAD model and littermate controls.Using ^{18}F-FDG PET/MRI and a published network analysis method, we conducted connectomic analyses in 5xFAD mice (n=64) and littermate controls (n=65) across 28 brain regions. Hierarchical modularization analysis was performed using adjacency matrices constructed by cross-correlations to yield primary and secondary modules, which were statically compared across age, sex, and genotype. Preliminary network analysis of the control group (male, WT, 4mo) revealed two primary modules within the whole brain network which were further partitioned into four secondary sub-modules of 5-10 brain regions/module. Upon reviewing brain regions within each sub-module for associated function(s), we noted module 1.1 represented predominately sensory (S) related function, modules 1.2 and 2.1 were mostly learning (L), while module 2.2 had a mixture of motor, perception, and sensory (MPS) associated brain regions. Statistical analysis of the four sub-modules were analyzed with age, sex, and genotype as the primary factors. Modules 1.1 (S) and 2.2 (MPS) showed a significant sex dependent effect for 12mo 5xFAD mice, significant effect of age in both sexes of WT mice between 4mo and 12mo, but only male 5xFAD mice had a significant effect between 6mo and 12mo. Interestingly, genotype was significant in strictly male 4mo mice for modules 1.1 (S), 2.1 (L), and 2.2 (MPS).These data illustrate the enhanced capacity of the new connectomic analysis approach to identify AD-related patterns both spatially and temporally using 18F-FDG PET data. Moreover, they replicate clinical manifestations of subjects with the same familial AD mutations, encouraging additional mechanistic studies to elucidate the mechanisms and etiology of this network connectomic phenomenon.

Disclosures: R.M. Silvola: None. **M. Veronese:** None. **S. Persohn:** None. **A.A. Bedwell:** None. **K. Eldridge:** None. **R. Speedy:** None. **P.R. Territo:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.04

Topic:

Support: NIH Grant UL1TR002529
NIA Grant RF1 AG051495
NIA Grant RF1 AG050597
NIA Grant U54 AG054345
NIA Grant K01 AG054753
NIA Grant R03 AG063250
NIH Grant NLM R01 LM012535

Title: Plcg2 is associated with the inflammatory response and is induced by amyloid plaques in alzheimer's disease

Authors: *E. J. MESSENGER[1], A. P.-Y. TSAI[1], C. DONG[1], P. B.-C. LIN[4], B. T. CASALI[1], M. MOUTINHO[4], Y. LIU[1], A. OBLAK[2], B. T. LAMB[4], G. E. LANDRETH[3], S. J. BISSEL[1], K. NHO[1];
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Abstract: Alzheimer’s Disease (AD) is a neurodegenerative disorder and the leading cause of dementia. It is characterized by amyloid beta (Aβ) plaque deposition, intracellular tau tangles, and microgliosis. Accumulating evidence from genetic studies suggest the importance of phospholipase C gamma 2 (PLCG2), an intracellular phospholipase that, in the brain, is found in microglia and an important downstream effector of immune receptors, in late-onset AD (LOAD) pathophysiology. However, the role of PLCG2 in AD is still poorly understood. Using bulk RNA-Seq (N=1,249) data from seven brain regions from the Accelerating Medicines Partnership-Alzheimer’s Disease Consortium (AMP-AD), we observed significant upregulation of PLCG2 expression in the parahippocampal gyrus, superior temporal gyrus, and inferior temporal gyrus in LOAD patients and a significant positive correlation of PLCG2 expression with amyloid plaque density. These findings from the human brain were recapitulated in the 5xFAD amyloid mouse model, which showed disease progression-dependent increases in Plcg2 expression with increased amyloid pathology. Furthermore, using bulk RNA-Seq data, we performed differential expression analysis by comparing cognitively normal older adults (CN) with high and low PLCG2 gene expression levels. Notably, genes related to inflammation and the inflammatory response were significantly altered between high and low PLCG2-expressing CN adults. These findings from human brain were recapitulated in mouse by generating mice with inactivated Plcg2 and by performing differential expression analysis comparing wild-type (WT) mice to mice lacking Plcg2 (Plcg2 inactivation), indicating that this model is appropriate to study PLCG2 as it relates to human disease. Additionally, PLCG2 co-expression network analysis of human MCI and AD microglia via single-cell RNA-Seq identified pathways closely

related to the inflammatory response, including regulation of I-kappa B/NF-kappa B signaling and response to lipopolysaccharide. These findings reinforce the importance of PLCG2 in the inflammatory response and suggest PLCG2 as an important mediator of the inflammatory processes underlying AD pathology. In conclusion, our results provide further evidence that PLCG2 plays an important role in AD pathophysiology and may itself be a potential target or be an important component of the pathways targeted in microglia-focused AD therapeutics.

Disclosures: E.J. Messenger: None. **A.P. Tsai:** None. **C. Dong:** None. **P.B. Lin:** None. **B.T. Casali:** None. **M. Moutinho:** None. **Y. Liu:** None. **A. Oblak:** None. **B.T. Lamb:** None. **G.E. Landreth:** None. **S.J. Bissel:** None. **K. Nho:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.05

Topic:

Support: NIH Grant UL1TR002529
NIA Grant RF1 AG051495
NIA Grant RF1 AG050597
NIA Grant U54 AG054345
NIA Grant K01 AG054753
NIA Grant R03 AG063250
NIH Grant NLM R01 LM012535

Title: Impact of PLCG2 Alzheimer's Disease Risk and Protective Variants on Microglial Biology and Disease Pathogenesis

Authors: *A. TSAI[1], P. B.-C. LIN[1], C. DONG[2], M. MOUTINHO[1], Y. LIU[2], A. OBLAK[3], S. J. BISSEL[5], K. NHO[2], B. T. LAMB[1], G. E. LANDRETH[4];
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Abstract: Alzheimer’s disease (AD) and other neurodegenerative diseases are typified by a robust microglial-mediated immune response. Genetic studies have demonstrated that variants in microglial genes are linked to risk for AD. Phospholipase C gamma 2 (PLCG2), whose variants confer altered risk for AD, is a critical signaling element for various immune receptors and is a key regulatory hub gene for immune signaling.GWAS studies demonstrated that the P522R variant (rs72824905) of PLCG2 was associated with reduced AD risk (OR= 0.68, p=5.83-10), and this variant is a functional hypermorph. Significantly, we identified a novel SNP

(rs617749044) associated with elevated AD risk (OR=1.164; p=0.047) encoding the PLCG2 M28L variant. However, a comprehensive analysis of PLCG2, whose genetic variants confer both protection and risk for AD, has yet to be explored in mouse models of AD.To investigate the impact of PLCG2 AD risk variants on microglia biology and disease pathology, we have generated mice bearing either the M28L risk or the P522R protective PLCG2 variant, all crossed onto the 5XFAD amyloidogenic murine model of AD.We provide data demonstrating that in individuals with AD, there is increased expression of PLCG2 in the brain, a finding that is recapitulated in 5XFAD mice. We show that PLCG2 variants program microglial phenotypes in 5XFAD mice, affect plaque pathology, and drive distinct transcriptional phenotypes of microglia in the presence of amyloid pathology. Compared to 5XFAD mice, behavioral analysis of 5XFAD; P522R mice demonstrated an improved performance in the Y maze assay, reflecting a restriction of cognitive decline. Our data suggest that the M28L variant had accelerated and exacerbated disease-related pathology. Conversely, the P522R variant appeared to attenuate disease severity and progression through an increased immune response program and enhance the clearance of amyloid plaques.Together, our findings provide evidence that PLCG2 plays an important role in AD pathophysiology, indicating PLCG2 as a potential new therapeutic target for AD.

Disclosures: A. Tsai: None. **P.B. Lin:** None. **C. Dong:** None. **M. Moutinho:** None. **Y. Liu:** None. **A. Oblak:** None. **S.J. Bissel:** None. **K. Nho:** None. **B.T. Lamb:** None. **G.E. Landreth:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.06

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

Support: NIH Grant U54 AG054349
NIH Grant U54 AG054345

Title: Late-onset AD risk mutation PICALMH458R prevents plaque generation in 5xFAD mice

Authors: *J. PHAN[1], K. M. TRAN[1], D. I. JAVONILLO[1], C. D. CUNHA[1], S. FORNER[1], S. KAWAUCHI[2], A. J. TENNER[1,3,4], F. LAFERLA[1,3], G. MACGREGOR[2,5], K. GREEN[1,3]; [1]Inst. for Memory Impairments and Neurolog. Disorders, [2]Transgenic Mouse Facility, [3]Neurobio. and Behavior, [4]Mol. biology and Biochem., [5]Dept. of Developmental and Cell Biol., Univ. of California, Irvine, Irvine, CA

Abstract: Alzheimer’s disease (AD) is an age-related neurodegenerative disorder characterised by the presence of amyloid-beta plaques and intraneuronal neurofibrillary tangles in the aged

brain. To understand the processes that drive late-onset AD genome-wide association studies (GWAS) have uncovered many risk loci. One of these is within the phosphatidylinositol-binding clathrin assembly protein (PICALM) gene, and a coding mutation has been identified (H458R) which conveys increased risk. CRISPR/Cas9 endonuclease-mediated genome editing was used to introduce the equivalent missense mutation in the C57BL/6J mouse genome (H465R). To explore how this risk mutation influences the development of AD relevant pathologies we crossed PicalmH465R mice with the 5xFAD mouse model of AD, to generate 4 groups (WT, PicalmH465R homozygous (HO), 5xFAD, and 5xFAD x PicalmH465R HO) and aged them to 4 months age, when plaque loads are being established. Coronal brain sections were immunolabeled with Thioflavin-S to visualize dense-core plaques in the hippocampus subiculum and cortex. No plaques were observed in wild-type C57BL/6J mice and PicalmH465R mice, as expected. PicalmH465R x 5xFAD mice exhibited significant reduction in plaque density in both brain regions compared to 5xFAD mice, as well as reductions in plaque volumes. Microglia numbers mirrored plaque load, with reduced microgliosis in 5xFAD x PicalmH465R mice compared to 5xFAD mice, due to the reduced plaque load. No changes in microglia and plaque interactions were seen. Soluble and insoluble Ab40 and Ab42 were measured in cortical and hippocampal brain homogenates. Similar to the observed plaque load, hippocampal insoluble fractions of 5xFAD x PicalmH465R have reduced levels of Aβ40 and Aβ42 compared to 5xFAD mice. A decrease was also observed in Aβ40 in 5xFAD x PicalmH465R cortical insoluble solution but no significant differences in Aβ42. Notably, soluble Abeta levels in the hippocampus were increased in 5xFAD x PicalmH465R mice, but decreased in the cortex, compared to 5xFAD mice. In summary, the results indicate PicalmH465R variant induces diminished Ab plaque burden in 5xFAD mice.

Disclosures: J. Phan: None. **K.M. Tran:** None. **D.I. Javonillo:** None. **C.D. Cunha:** None. **S. Forner:** None. **S. Kawauchi:** None. **A.J. Tenner:** None. **F. LaFerla:** None. **G. MacGregor:** None. **K. Green:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.07

Topic:

Support: NIH U54 AG054345

Title: Characterization of functional enhancer variants associated with Alzheimer’s disease

Authors: *C. PREUSS[1], X. CHEN[2], G. CARY[1], R. PANDEY[1], K. KOTREDES[1], D. GARCEAU[1], C. THEESFELD[2], P. SHANNON[3], A. UYAR[1], G. R. HOWELL[1], M. SASNER[1], C. C. FUNK[3], O. TROYANSKAYA[4], G. CARTER[1];

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Abstract: Background: GWAS variants associated with late-onset Alzheimer's disease (LOAD) are enriched in noncoding regions of the genome, with a large proportion of functional variants falling into enhancers. Yet, identifying causal enhancers and the cell types in which they are active remains challenging. Functional conservation between human and mouse can reveal key transcriptional and chromatin state dynamics linked to enhancer function. **Methods:** We developed a novel multi-tier approach to assess functional enhancer conservation by leveraging chromatin, ChIA-PET and transcriptomic data sets from human and mouse to provide a comprehensive catalog of LOAD enhancers. Further, ATAC-seq data from human and mouse was integrated to predict cell-type specific effects of the prioritized regulatory elements. Then, two deep learning approaches (DeepSEA, TReNA) were implemented to identify regulatory variants in enhancers that are predicted to disrupt expression of nearby candidate genes. Finally, the top-conserved disease-associated variants were used to create five novel enhancer mouse models as part of the MODEL-AD initiative. **Results:** Heritability analysis across multiple neurodegenerative disease traits revealed a specific enrichment of LOAD GWAS variants within lowly conserved enhancer elements active in microglia. We also identified a significant enrichment of an oligodendrocyte and microglia signature (FDR $p < 0.5$) across conserved LOAD enhancers. Among microglia specific enhancers, binding sites of transcription factors (TF) linked to inflammation and microglia activation (SPI1, RUNX1, NFKB) were over-represented while oligodendrocyte enhancers showed an enrichment for a TF (MYC) associated with cell proliferation. By integrating transcriptome data from post-mortem brains, we found that microglia enhancers harboring SPI1 binding sites are linked to increased expression of genes mediating an inflammatory response across species. Our TReNA approach identified variants in *PTK2B* which alter binding sites for two TF (RARA,CUX2) that showed a high correlation with *PTK2B* expression. To functionally follow-up on our findings, we created enhancer deletion and knock-in models for LOAD variants in five well-known GWAS loci (*ADAMTS4*, *BIN1*, *PTK2B*, *SCIMP*, *EPHA1*). These variants are predicated to mediate the expression of nearby candidate genes in a cell type-specific manner. We provide initial functional characterization for our novel models based on brain RNA-seq data. **Conclusion:** Our findings provide not only a translational catalog of cell type-specific LOAD enhancers but also a novel set of five mouse models to study their effects *in vivo*.

Disclosures: C. Preuss: None. **X. Chen:** None. **G. Cary:** None. **R. Pandey:** None. **K. Kotredes:** None. **D. Garceau:** None. **C. Theesfeld:** None. **P. Shannon:** None. **A. Uyar:** None. **G.R. Howell:** None. **M. Sasner:** None. **C.C. Funk:** None. **O. Troyanskaya:** None. **G. Carter:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.08

Topic:

Support: NIA U54 AG054345
NIA R01 AG055104

Title: Incorporating genetic diversity to improve alignment of mouse models to human Alzheimer's disease

Authors: *K. J. ELK, A. UYAR, D. GARCEAU, D. BEDNARCZYK, R. O'ROURKE, K. KOTREDES, M. SASNER, G. W. CARTER, G. R. HOWELL;
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Abstract: A growing body of research is revealing the benefits of incorporating genetically diverse mouse strains to better model human Alzheimer's disease (AD). For instance, mouse models of AD on genetically distinct wild-derived mouse strains demonstrated robust differences in immune response to amyloid and neurodegeneration, better representing the phenotypic spectrum of AD than previous models. To further investigate the importance of genetic diversity, we are generating AD-relevant mouse models on at least ten Collaborative Cross (CC) strains, a recombinant inbred mouse panel created from eight highly diverse founder strains. To date, C57BL/6J (B6J) mice homozygous for humanized APOE4 allele and carrying mutant APP and PS1 transgenes (APPswe, PS1de9) have been crossed to five CC strains that were selected to maximize variation in AD-relevant genes, including *Trem2* and *Cd33*. Cohorts of male and female B6JCCnF1.APOE4.APP/PS1 mice and controls (6/sex/strain/Tg-genotype) were aged to 8 months (a midpoint for amyloid deposition in B6.APP/PS1 mice) and brain hemispheres were processed for transcriptional profiling by RNA-seq and multiple neuropathological assessments (including amyloid deposition, neurodegeneration, and glial activation) via immunohistochemistry (IHC). Data were assessed for strain-, genotype- and sex-specific variation. RNA-seq analysis revealed up-regulation of neuroinflammation related pathways (lysosome, complement and coagulation cascades) in all CC strains as an effect of mutant APP and PS1 transgenes in presence of APOE4 allele. On the other hand, we observed significant CC strain- and sex- specific variation in immune (Fc gamma R-mediated phagocytosis) and metabolic (glycosphingolipid biosynthesis) response to amyloid pathogenesis. Alignment with human AD signatures revealed high strain- and sex- specific variation in correlation with synaptic signaling, cell cycle and DNA repair related gene modules. Preliminary IHC data revealed marked differences in amyloid deposition across the CC strains. For instance, in the presence of the amyloid drivers, B6J/CC037F1 and B6J/CC006F1 exhibited elevated levels of parenchymal plaque compared to B6J. Also, vascular amyloid, indicative of cerebral amyloid angiopathy (CAA), varied across the strains, independent of parenchymal amyloid load, with B6J/CC037F1 and B6J/CC002F1 showing the highest levels of CAA. Assessments of neurodegeneration and glial activation are in progress. Overall, this study further supports the incorporation of genetically diverse mouse strains, including CC strains, to better model complex neurodegenerative disorders such as AD.

Disclosures: K.J. Elk: None. **A. Uyar:** None. **D. Garceau:** None. **D. Bednarczyk:** None. **R. O'Rourke:** None. **K. Kotredes:** None. **M. Sasner:** None. **G.W. Carter:** None. **G.R. Howell:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.09

Topic:

Support: NIH Grant U54AG054345

Title: The MODEL-AD Explorer: An open access resource for comparing phenotypic data from mouse models of Alzheimer's disease

Authors: *A. GREENWOOD[1], S. GELFAND[1], R. PANDEY[2], N. REZAIE[3], A. L. OBLAK[4], S. FORNER[3], A. UYAR[2], J. MINCER[5], M. FAZZA[1], M. A. PETERS[1], R. YAXLEY[1], A. VANDER LINDEN[1], J. SCHNEIDER[1], A. MORTAZAVI[3], L. MANGRAVITE[1], F. LA FERLA[3], A. TENNER[3], B. LAMB[4], K. N. GREEN[3], G. CARTER[2];
[1]Sage Bionetworks, Seattle, WA; [2]The Jackson Lab., Bar Harbor, ME; [3]Univ. of California, Irvine, Irvine, CA; [4]Indiana Univ., Indianapolis, IN; [5]Univ. of Utah, Salt Lake City, UT

Abstract: Selecting an optimal mouse model for target validation experiments and preclinical testing of compounds remains a major barrier to the progression of basic research discoveries through the Alzheimer's disease (AD) drug development pipeline. There are numerous challenges that contribute to the inability to identify ideal model systems, including lack of standardized phenotypic measures, lack of mice that fully replicate features of late-onset human disease, and licensing requirements, among others. To address these issues, the Model Organism Development and Evaluation for Late-onset Alzheimer's Disease (MODEL-AD) consortium has generated dozens of new mouse models of late-onset, sporadic AD that are available without restriction to the research community. These strains were developed using a variety of strategies to model AD, including introduction of human AD genetic variants, AD risk gene knockouts, and knockins of humanized AD related genes. All MODEL-AD models have been characterized using a rigorous, standardized phenotypic pipeline across multiple timepoints, and all data is freely available through the AD Knowledge Portal. To facilitate evaluation of these models by interested researchers, we have developed the MODEL-AD Explorer (https://modeladexplorer.org/). The Explorer currently hosts pathology and gene expression data from 15 unique models. The pathology explorer presents quantification of amyloid plaques, microglia, astrocytes, and dystrophic neurites based on immunostaining as well as soluble and insoluble amyloid-beta levels in brain tissue. A second explorer allows exploration of gene expression data for a subset of models and can be used in two ways: 1) viewing all differentially

expressed genes for selected mouse models as a function of age and sex or 2) comparing differential expression of selected genes across models, sexes, and ages. A third explorer shows the extent to which gene expression changes in mouse models mimic expression changes in humans with AD. Once users identify a model of interest based on phenotypic evaluation, they can visit the Jackson Laboratory website to learn more about each strain. The MODEL-AD Explorer will continue to grow to house phenotypic data from additional MODEL-AD developed models as well as new data modalities.

Disclosures: A. Greenwood: None. **S. Gelfand:** None. **R. Pandey:** None. **N. Rezaie:** None. **A.L. Oblak:** None. **S. Forner:** None. **A. Uyar:** None. **J. Mincer:** None. **M. Fazza:** None. **M.A. Peters:** None. **R. Yaxley:** None. **A. Vander Linden:** None. **J. Schneider:** None. **A. Mortazavi:** None. **L. Mangravite:** None. **F. La Ferla:** None. **A. Tenner:** None. **B. Lamb:** None. **K.N. Green:** None. **G. Carter:** None.

Poster

P197. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VI

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P197.10

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

Support: 943450GA45U

Title: Phenotypic characterization of UCI MODEL-AD mice using RNA-seq

Authors: *M. GANTUZ, H. Y. LIANG, N. REZAIE, G. BALDERRAMA-GUTIERREZ, C. MODEL-AD, A. MORTAZAVI;
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Abstract: Phenotypic characterization of UCI MODEL-AD mice using RNA-seqAuthors: Magdalena Gantuz, Heidi Y. Liang, Narges Rezaie, Gabriela Balderrama-Gutierrez,MODEL-AD Consortium, Ali MortazaviThe development and characterization of new Alzheimer’s Disease (AD) mouse modelsare critical for the understanding the progression of the pathology and as a platform for theevaluation of new drugs. The UCI MODEL-AD project focuses on the development of late-onsetAD (LOAD) mouse models based on human GWAS data. We evaluate each new modeldeveloped in our group by performing a comparative transcriptomic analysis of cortex andhippocampal tissues in order to relate changes in expression to other phenotypic changes.As part of our standard pipeline, we test each new GWAS variant model at the age of 4months crossed with 5XFAD mice to regular 5XFAD mice and GWAS variant-only mice.Differential gene expression analysis allows us to quantify the impact of the variant on thepathology. We discuss the result of several of our latest models data. RNA

transcriptomeanalysis combined with phenotypic characterization is fundamental to the identification of themost appropriate LOAD mouse model.

Disclosures: M. Gantuz: None. **H.Y. Liang:** None. **N. Rezaie:** None. **G. Balderrama-Gutierrez:** None. **C. Model-ad:** None. **A. Mortazavi:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.01

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

Support: NIH Grant NS104147

Title: Impact of Non-Pharmacological Chronic Hypertension on a Transgenic Rat Model of Cerebral Amyloid Angiopathy (rTg-DI)

Authors: *A. STANISAVLJEVIC[1,2], J. M. SCHRADER[1,2], X. ZHU[1,2], J. GUILMAIN[2], A. HANKS[1,2], M. MAJCHRZAK[1,2], J. K. ROBINSON[1,2,3], W. E. VAN NOSTRAND[1,2];
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Abstract: Cerebral Amyloid Angiopathy (CAA), a common comorbidity of Alzheimer's disease (AD), is a small vessel disease characterized by deposition of fibrillar amyloid β (Aβ) in and around blood vessels of the brain, neuroinflammation, cognitive impairment and dementia. Hypertension, another prominent small vessel disease, has been found to increase risk of dementia and may worsen associated pathologies but clinical data regarding its effects in CAA patients is controversial. Studies have reported that hypertension can worsen CAA related intracerebral hemorrhage (ICH), have little to no effect or even lessen CAA related ICH. To understand effects of hypertension on pathologies of CAA, we bred rTg-DI transgenic rats, a model of CAA, with spontaneously hypertensive, stroke prone rats (SHR-SP) producing rTg-DI/SHR-SP and non-transgenic SHR-SP littermates (n= 6-7 including males and females). Also utilized in this study were rTg-DI rats and non- transgenic (WT) littermates (n= 6-7 including males and females). Blood pressure measurements and behavioral testing were completed at 7 months of age before euthanization and brain tissue collection. Results showed that both rTg-DI/SHR-SP and SHR-SP littermates exhibit elevated blood pressures. However, behavioral testing showed no changes between rTg-DI/SHR-SP and rTg-DI rats. Forebrain tissue was assessed for known pathologies of CAA including vascular amyloid load, vessel occlusions, microbleeds, and microglial cell activation. Vascular amyloid load in cortex, thalamus and surface pial vessels was significantly increased in rTg-DI/SHR-SP rats compared to rTg-DI rats. However, the number of thalamic vessel occlusions were no different between rTg-DI and rTg-

DI/SHR-SP rats. Further, no difference in microbleeding was observed between SHR-SP, rTg-DI, and rTg-DI/SHR-SP animals. Our findings suggest that hypertension causes modest impacts on CAA pathologies in 7 month-old rats and provide a non-pharmacological model to further study hypertension and CAA as co-morbidities for cerebral small vessel disease.

Disclosures: A. Stanisavljevic: None. **J.M. Schrader:** None. **X. Zhu:** None. **J. Guilmain:** None. **A. Hanks:** None. **M. Majchrzak:** None. **J.K. Robinson:** None. **W.E. Van Nostrand:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.02

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

Support: NIH grant AG055104
Alzheimer's Association Neuroimmune grant
Diana Davis Spencer Foundation

Title: WSB/EiJ — A genetic context to determine vascular contributions to cognitive decline and dementia

Authors: *G. HOWELL[1], *G. HOWELL[1], H. YANG[1], K. KEEZER[1], A. UYAR[1], G. CARTER[1], M. SASNER[1], H. H. PATEL[3], A. E. WALKER[4], P. R. TERRITO[5], K. D. ONOS[2]; [2]Res., [1]The Jackson Lab., Bar Harbor, ME; [3]Univ. of California San Diego, San Diego, CA; [4]Univ. of Oregon, Eugene, OR; [5]Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Vascular contributions to cognitive decline and dementia (VCID) are now widely appreciated but the underlying genetic causes remain elusive. Current studies relevant to VCID use mouse models focused on environmental risk factors such as diet-induced obesity. To complement these approaches, we are taking advantage of mouse genetic diversity to understand the mechanisms underlying VCID.
We performed a strain survey using amyloid drivers APP^{swe} and $PSEN1^{de9}$ (*APP/PS1*). *APP/PS1* transgenes were backcrossed from C57BL/6J (B6) to founder strains of the Collaborative Cross (129S1/SvlmJ, NOD/ShiltJ, NZO/HILtJ, WSB/EiJ, CAST/EiJ and PWK/PhJ), NSG (NOD-skid-gamma), and DBA/2J. Cerebral amyloid angiopathy (CAA) was used as a VCID-relevant phenotype that is largely absent in B6.*APP/PS1* mice. CAA is found in 85-95% of AD cases, positioning CAA as one of the strongest vascular contributors to age-related cognitive decline. Of the nine strains assessed, WSB showed the greatest propensity and amount of CAA. To understand the increased susceptibility to CAA, cohorts of male and female WSB.*APP/PS1* and

WSB mice were aged from 4 to 18 months (mos). Vascular amyloid deposits were observed as early as 8 mos in cortical and leptomeningeal vessels. These vascular amyloid deposits colocalized with GFAP-positive astrocyte processes but were devoid of microglia and collagen. Brain transcriptional profiling comparing WSB.APP/PS1 to WSB mice at multiple ages identified genes relating to vascular health including basement membrane, endothelial function, and blood vessel formation. These genes were not differentially expressed in similar studies performed in B6 mice, supporting our hypothesis that WSB are prone to CAA due to inherent vascular-dependent dysfunction. Supporting our hypothesis, Prussian blue staining revealed the presence of microbleeds in aged WSB mice – even in the absence of amyloid. To validate these findings, the middle cerebral artery is being isolated from aging WSB mice and endothelial cell function assessed. Preliminary data suggest that deficits in mitochondria function may underlie vascular dysfunction. High-resolution respirometry indicated a significant depression of mitochondria respiration in the hippocampus from 4 mos WSB.*APP/PS1* mice, prior to observed CAA.
We are also exploring the utility of WSB as a preclinical model for VCID using more clinically relevant genetic (humanized amyloid and tau, allelic series of apolipoprotein E) and environmental (high fat/high sugar diet) risk factors. We are also using WSB to improve the face validity of other complex neurodegenerative disorders, including retinal vasculopathies and glaucoma.

Disclosures: G. Howell: None. **H. Yang:** None. **K. Keezer:** None. **A. Uyar:** None. **G. Carter:** None. **M. Sasner:** None. **H.H. Patel:** None. **A.E. Walker:** None. **P.R. Territo:** None. **K.D. Onos:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.03

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

Support: DFG Grant SFB 1089
DFG Grant SPP1757
DFG Grant INST1172 15
DFG Grant DI853/3-5&7
The BONFOR program of the Medical Faculty of the University of Bonn
Alexander von Humboldt Foundation, Georg Forster postdoctoral fellowship

Title: Two-photon imaging identifies blood-brain barrier alterations in a murine Alzheimer’s disease model

Authors: A. HANAFY[1,2], P. STEINLEIN[1,3], I. PAULUßEN[3], N. S. VANA[3], A. LAMPRECHT[1], D. DIETRICH[3];
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Abstract: Since the identification of Alzheimer's disease (AD) a century ago, inconclusive data have been suggesting three hypotheses of pathogenesis involving the blood-brain barrier (BBB): disrupted tight junctions (TJ), decreased functionality of efflux transporters and increased activity of receptor for advanced glycation end products (RAGE), as drawn from different BBB models that favor controllability and accessibility over biological validity. In this study, a newly established BBB model was used to investigate the validity of these hypotheses in transgenic *Tg2576* AD mice. This model utilizes a novel controlled micropipette-based local perfusion of capillaries in acute brain slices, while monitoring the cellular transport of test compounds in real-time with high resolution two-photon (2P) microscopy. Acute cortical brain slices were isolated from age-matched wildtype and transgenic *Tg2576* mice, and placed in a submerged chamber of a 2P microscope. A blood vessel was pierced with a dye-filled pipette and ejected under 100 mmHg pressure. The TJ integrity was functionally and structurally assessed by quantifying the extravascular leakage and membrane diffusion of injected dyes and 3D reconstructed focused ion-beam scanning electron microscopy (FIB-SEM). The functionalities of two ATP-binding cassette (ABC) transporters, ABCB1 and ABCC1, and RAGE receptor were evaluated as well. Compared to wildtype mice, the paracellular diffusion of tetramethylrhodamine was doubled in Tg2576 capillaries, while the membrane diffusion of FM1-43 probe from the luminal to abluminal side of endothelial cells was 2.3-fold higher. FIB-SEM analysis revealed scarcer and shorter TJ in *Tg2576* capillaries. Based on the endothelial accumulation of calcein dye under sole or combined inhibition of ABCB1 and ABCC1 transporters, their efflux activity in *Tg2576* capillaries decreased by 30% and 85%, respectively. Surprisingly, exogenous β-amyloid endothelial uptake from blood to brain was 60% lower in *Tg2576* capillaries compared to wildtype mice, regardless of RAGE inhibition by FPS-ZM1. The results may imply TJ disruption possibly due to partial disassembly of the transmembrane domains of TJ proteins. The reduced efflux activity of ABCB1 and ABCC1 transporters could account for the poor β-amyloid clearance from AD capillaries. However, as opposed to the often-reported data, RAGE receptor is not overly activated in *Tg2576* capillaries.

Disclosures: A. Hanafy: None. **P. Steinlein:** None. **I. Paulußen:** None. **N.S. Vana:** None. **A. Lamprecht:** None. **D. Dietrich:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.04

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

Support: NIH grant 3R15HL140523-01S1

Title: Primary cerebrovascular cilia are involved in blood pressure and Alzheimer's disease manifestations through ciliary muscarinic acetylcholine receptors.

Authors: K. V. FORERO[1], W. M. MESSER, Jr[1], ***W. ABOUALAIWI**[2];
[1]Pharmacol. and Exptl. Therapeut., Univ. of Toledo, Toledo, OH; [2]Pharmcology and Exptl. Therapeut., Univ. of Toledo/College of Pharm. and Pharm, Toledo, OH

Abstract: Cilia dysfunctions manifest a broad range of symptoms, including hypertension (HTN) and cognitive and memory dysfunction - symptoms also observed in Alzheimer's disease (AD). We demonstrated that the inability of primary cilia to sense and transmit fluid shear stress can lead to nitric oxide (NO) deficiency and cause vascular HTN. It is well documented that decreased biosynthesis of NO contributes to cerebral amyloid angiopathy in AD patients through increased deposition of beta amyloid (Aβ). Disruption of cholinergic neurotransmitter pathways, which are important for cognition, memory and learning abilities has been reported in AD patients. Muscarinic acetylcholine receptors (mAChRs) are also expressed throughout the cardiovascular system and can regulate BP. However, the connection between cilia and mAChR signaling in the brain has never been investigated before. Supported by our recent discovery of the mAChRs (M1 and M3) localization to primary cilia, we propose a **bold idea to look at the pathophysiological roles of cerebrovascular ciliary receptors in BP and AD.** We generated vascular-specific *AChM3R* and *Tg737* (important for ciliogenesis) knockout mouse lines. These mice developed high BP. We formulated the hypothesis that *ciliary mAChRs in the cerebrovascular endothelial cells contribute to AD progression through diminished NO biosynthesis*. We used human brain endothelial cells (HBECs) to show that AChM3R, its downstream effector PIP2, and eNOS localize to primary cilia. We also discovered that mAChR-specific activation (with CDD-0102A) increases cilia length in HBECs and cilia formation in cilialess *Tg737* cells and was also associated with an increase in eNOS activity (p-eNOS). We set out to determine if Aβ affects primary cilia structure and potentially leads to cilia sensory dysfunction and impaired ciliary AChM3R signaling. We showed that exposure to 2.5 μM synthetic human Aβ42 resulted in significant reduction in primary cilia length and ciliation percentage. We further demonstrated that upon RNAi-mediated knockdown of Tulp3, a significantly decreased trafficking of AChM3R to the cilia was detected with no effect on its plasma membrane localization. To further confirm if ciliary AChM3R localization is necessary for its activity, we treated our cilialess *Tg737* cells with AChM3R agonists (cevimeline) or antagonist (difenidol) and assayed for AChM3R activity using the arachidonic acid release assay. Interestingly, AChM3R activity did not change in cilialess *Tg737* cells following cevimeline or difenidol treatment. The proposed studies offer advanced scientific understanding of primary cilia in cerebrovascular and cognitive disease.

Disclosures: K.V. Forero: None. **W.M. Messer:** A. Employment/Salary (full or part-time):; Dr. Messer is the founder and president of Psyneurgy Pharmaceuticals LLC.. **W. Aboualaiwi:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.05

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

Title: Abeta peptides disrupt the barrier integrity and glucose metabolism of human induced pluripotent stem cell-derived brain microvascular endothelial cells

Authors: *S. RAUT[1], L. CUCULLO[1], A. AL-AHMAD[2];
[1]Oakland Univ. William Beaumont Sch. of Med., Rochester, MI; [2]Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: Amyloid beta peptides are important components of Alzheimer's disease and cerebral amyloid angiopathy, and they have been linked to negative effects on the blood-brain barrier (BBB) in *in vivo*. The physiological and molecular mechanisms by which these peptides affect the brain vasculature, however, are unknown. The goal of this work was to see how Aβ peptides affected the cellular response of iPSC-derived brain microvascular endothelial cells (BMECs). In this model, changes in barrier function, efflux transporter activity, glucose absorption, and metabolism were evaluated. Although iPSC-derived BMECs sustained prolonged exposure (<72 hours) to a high level of Aβ peptides including Aβ42, such cells also suffered from a loss of barrier integrity, coupled with reduced glucose uptake and impaired bioenergetics activity. Overall, our research demonstrates the capacity of iPSC-derived BMECs to replicate features seen in other models and reveals that Aβ peptides may undermine the BBB via different targets. Our previous investigation, which used iPSC-derived BMECs from two patients with a Familial type of Alzheimer's disease, found a similar altered bioenergetics profile. To summarize, this study shows that iPSC-derived BMECs generated using the Shusta technique can exhibit behavior and cellular response similar to that shown in the literature employing somatic cells or in vivo models.

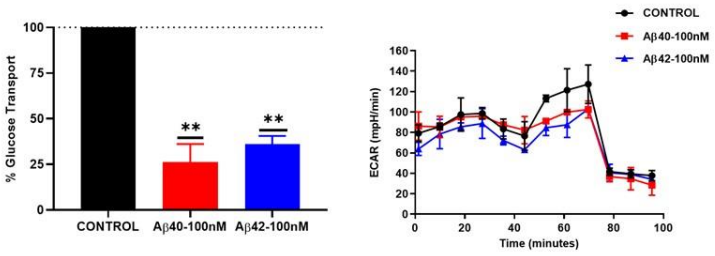


Figure 1. Aβ peptides impact glucose transporters expression and transport and impact cellular energetic profile.

Disclosures: S. Raut: None. **L. Cucullo:** None. **A. Al-Ahmad:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.06

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

Support: AG042178
AG047812
NS105473
AG060767
AG069333
AG066347

Title: Increased levels of SARS CoV-2 ACE2 receptors in an age-dependent manner in Alzheimer’s and Huntington’s mouse models

Authors: *J. LOEWEN, S. UPPALA, E. ORLOV, H. MORTON, N. SAWANT, M. VIJAYAN, H. REDDY;
Texas Tech. Univ., Lubbock, TX

Abstract: Abstract: The purpose of our study is to assess the levels of angiotensin-converting enzyme 2 (ACE2) in mouse models of Alzheimer's disease and Huntington's disease. The SARS CoV-2, commonly known or COVID-19, has been the cause of a global outbreak since the first reported case in China in late 2019. The virus is spread directly person-to-person via respiratory droplets and utilizes receptors such as the angiotensin-converting enzyme 2 (ACE2) to enter host cells. SARS CoV-2 is most known for the severe respiratory distress it can cause, most often affecting individuals who are older as well as those with other comorbidities, such as Alzheimer's, Huntington's, diabetes, and obesity. Additionally, neurological manifestations have also been reported in those infected with Covid-19. These include neurodegenerative disorders which also have correlations to the ACE2 levels, also suggesting a connection to increased susceptibility to SARS CoV-2. Our initial investigation makes use of mice models, specifically the cerebellum, to understand the correlation between age and ACE2 levels. It is believed that there is an upregulation of the ACE2 receptor levels with age and age-related neurological diseases such as Alzheimer's and Huntington's. **Methods:** Using brain tissues from 2-, 6-12- and 20-month-old wild-type (WT) and also age-matched transgenic mouse models of Alzheimer's disease (APP and Tau) and Huntington's disease (BACHD and HD-knockin), we measured levels of ACE2. Results: We found age-dependent significantly increased levels of ACE2 in WT, APP, Tau BACHD and HD-knockin mice. Our comparative analysis of 1) WT to APP, 2) WT to Tau, 3) WT to BACHD mice revealed that ACE2 levels were increased in APP, Tau, BACHD and HD-knockin mice. These observations suggest that ACE2 levels increased with disease progression in Alzheimer's disease and Huntington's disease in an age-dependent manner. **Conclusions:** Our study findings suggest that age and neurodegenerative diseases result in increased susceptibility to acquiring Covid-19.

Disclosures: J. Loewen: None. **S. Uppala:** None. **E. Orlov:** None. **H. Morton:** None. **N. Sawant:** None. **M. Vijayan:** None. **H. Reddy:** None.

Poster

P215. APP/Amyloid and The Brain Vasculature

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P215.07

Topic: H.08. Learning and Memory

Support: NIH R21AG069140-01

Title: Branched-chain amino acid (BCAA) restriction improves cognition and systemic metabolism in APP/PS1 mouse model for Alzheimer's disease

Authors: *M. SIDDIK[1], H. SHAH[1], R. GANNABAN[1], V. HEGDE[1], A. C. SHIN[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by accumulated amyloid (Aβ-42) plaques, neurofibrillary tangles, and diminished neurotransmitters in the brain. Recently, our lab has shown high circulating branched-chain amino acids (BCAAs) in both AD humans and animals, most likely due to impaired BCAA degradation. High BCAAs can potentially lead to neurotransmitter imbalance, reduction in nerve growth factor, glutamate-induced excitotoxicity, and insulin resistance through mammalian target of rapamycin. Importantly, all these consequences represent the key pathological features of AD, making BCAAs a potentially significant contributor to this disease. Therefore, we tested if restriction of dietary BCAAs can ameliorate AD pathogenesis in transgenic AD mice. 10-12 months old WT or APP/PS1 mice were placed on either a chow diet (CD) or a CD with 50% BCAA restriction for two months. The BCAA-restricted diet was adjusted for all other amino acids to make it iso-caloric and iso-nitrogenous compared to the CD. For assessing cognitive function, Y-maze and novel object recognition (NOR) tests were used at baseline and after BCAA restriction. Animals were sacrificed after 5 hours of fasting, blood and tissues were collected for analysis. Baseline and post-restriction blood glucose, insulin, and BCAAs were also measured. To evaluate changes in neuropathological hallmarks of AD following dietary BCAA restriction, cortical protein expression of pTau and Aβ-42 were measured by western blot.BCAA restriction lowered plasma insulin levels in APP/PS1 mice compared to chow-fed animals, indicating a possible improvement in insulin sensitivity. This phenomenon was further supported by a reduction in blood glucose. While there was no difference in NOR results between groups, AD mice on a BCAA-restricted diet showed improved cognition as evidenced by a higher % of spontaneous alternations in Y-maze compared to both their baseline and chow-fed group. Surprisingly, we did not observe any changes in the amount of Aβ-42 in the cortex upon BCAA restriction. Our findings suggest that two months of BCAA restriction is able to lower fasting blood glucose, insulin, and improve cognition in AD mice. Considering that a pre-diabetic glucose dysregulation and insulin resistance are associated with AD development, our results suggest a possibility that BCAA restriction may improve cognitive function partly via correcting the metabolic impairments. To understand the underlying mechanisms for the improved cognition, we plan to examine neurotransmitter synthesis, autophagy, and mitochondrial and oxidative stress markers in the brain.

Disclosures: M. Siddik: None. **H. Shah:** None. **R. Gannaban:** None. **V. Hegde:** None. **A.C. Shin:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: C. Gus Glasscock, Jr. Endowment Fund 2022

Title: Exposure to combustion-derived air pollution may induce prolonged neuroinflammation

Authors: *S. H. PRADHAN[1], M. GIBB[2], C. M. SAYES[1];
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Abstract: Combustion-derived air pollution is a multi-layered environmental toxin which is becoming a global health concern due to rapid urbanization. Air pollution contains mixtures of pro-inflammatory stimulants such as fine and ultra-fine particulate matter ($PM_{<2.5}$), gases (CO and NOx), and metals (Mn). Inhalation of these compound are linked to systemic damage through the peripheral circulatory system from the lungs. This systemic damage implicates the central nervous system, due to co-morbidity of disease link to environmental air pollution. In particular air pollution is linked to neuroinflammatory effects as well as prolonged damage with neurodegeneration. This specific study looks at an *in vitro* model of an inhalation exposure to combustion-air pollution, specifically for diesel particulate matter (DPM) on alveolar macrophages and collecting the post-exposure serum to induce activation of microglial cells. For this study, alveolar macrophages were exposed to aerosolized DPM in an acute exposure (24-hour incubation) and tested for the expression of MCP-1, IL6, and TNF-α. Once release of cytokines and chemokines was confirmed, post-exposure serum was collected from cultured alveolar macrophages and introduced to a healthy culture of microglial cells (HMC3) over 72-hours and tested for activation (HLA-DR and CD14 expression). Lipopolysaccharides (LPS; 10ng/mL) was used as a positive control to stimulate activation of microglial cells. Once activation was confirmed cells were tested for ROS generation and neurotoxic cytokine expression.
Microglial cells, through a feedback loop with surrounding neurons, release neurotoxic pro-inflammatory markers, these include, IL1β, IL6, TNF-α and CD86 and cause internal damage to neurons. Results indicated high expression of ROS measured through H2O2 and observed with confocal fluorescence microscopy. There was moderate expression of IL1β, IL6, TNF-α and CD86 through exposure. This confirmed activated microglial cells release inflammatory markers which could implicate the indirect pathway of exposure (through lungs) to the brain as a method of inducing neuroinflammation and possibly neuronal cellular damage.

Disclosures: S.H. Pradhan: None. **M. Gibb:** None. **C.M. Sayes:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work has received funding from Co-Create Knowledge for Pharma Innovation with Takeda (COCKPI-T®).

Title: Establishment and comparison of microglia/astrocyte/neuron co-cultures derived from rodent spinal cord and cortical preparations for the study neuroinflammatory processes in vitro

Authors: *D. AMADIO[1], C. NODIN[1], M. SVEDMAN[1], J. SJOHOLM[1], S. ILLES[2]; [1]Cellectricon AB, Mölndal, Sweden; [2]Inst. of Neurosci. and Physiol., Gothenburg Univ., Gothenburg, Sweden

Abstract: Increasing evidence supports inflammation in the central nervous system (CNS) as a major player in the pathogenesis of human neurodegenerative disease. Microglia, the resident macrophages of the central nervous system, have a central role in maintaining cellular homeostasis; in contrast, microglial dysfunction has been linked to CNS disease progression. To best study microglia in a CNS physiologically relevant context, an *in vitro* cell culture systems comprised of microglia, astrocytes and neurons should be used, with the aim to dissect the cellular effect of microglia on neuronal activity and the cross-talk along the neuroinflammatory axis. In this study, we aim to develop two different systems that will allow the *in vitro* study of neuroinflammatory processes in complex primary rodent cell culture in the two different components of the CNS: brain and spinal cord. We have optimized two *in vitro* co-culture systems comprised of microglia, astrocytes and neurons in both mouse cortex and rat spinal cord. These can be used to investigate the role of (activated) microglia in the pathogenesis and progression of neurodegenerative disorders. We characterized the established models by using high content imaging and analysis. In addition, we further assessed neuronal function using Cellectricon's proprietary optical electrophysiology platform, and we finally examined the effect of LPS stimulation on microglia and neuronal function. The optimized microglia/astrocyte/neuron models permit robust survival of microglia *in vitro* in the presence of astrocytes and neuronal cells, and microglia survival can be further modulated by controlling culture conditions. Remarkably, we show that these co-culture systems have a healthy neuronal network and preserved neuronal activity as indicated both by electric field stimulation evoked responses and spontaneous activity patterns. We have also highlighted the difference between the two models (mouse cortex vs rat spinal cord) using the optimized co-culture condition and how they responded differently to culture stimulation *in vitro*. Given the results obtained in this study, the presented models are excellent tools suitable to study neuroinflammation *in vitro*, showing that a functionally active neuronal network can be achieved under culture condition that also permits robust microglia survival. Furthermore, the models are established in a high density 384-well format and are thus well suited for usage in medium throughput screening applications e.g. to study neuroinflammation in neurodegeneration and neuropathic pain.

Disclosures: D. Amadio: None. **C. Nodin:** None. **M. Svedman:** None. **J. Sjoholm:** None. **S. Illes:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Grant FDN 167270
MSSOC Grant 3527
CIHR Postdoctoral Fellowship

Title: Perturbators of microglia functions with ageing: Identification of osteopontin through single cell and spatial RNA sequencing

Authors: *Y. DONG, R. W. JAIN, B. M. LOZINSKI, C. D'MELLO, F. VISSER, S. GHORBANI, V. YONG;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Oxidized phosphatidylcholine (OxPC) species formed in multiple sclerosis (MS) lesions are proinflammatory and neurotoxic. Microglia depletion exacerbates OxPC mediated neurodegeneration, suggesting they are important for OxPC clearance (Dong et al., Nature Neurosci 2021). Since aging alters microglia transcriptomic signatures and functions, we aimed to determine if and how microglia responding to OxPC mediated neurodegeneration becomes dysfunctional due to aging by comparing OxPC-induced lesions in the spinal cords of 6-week and 52-week-old mice. Histology and immunofluorescence showed OxPC-induced lesions in spinal cord white matter was more severe in 52-week compared to 6-week-old mice with greater total lesion volume and axonal loss, and reduced oligodendroglial counts. Single cell RNAseq of live cells sorted from homogenized spinal cords identified multiple distinct microglia/macrophage clusters and there was upregulation of aging signatures such as interferon signaling and antigen presentation genes in the healthy 52-week spinal cord compared to the 6-week samples. Moreover, microglia/macrophages in the 52-week spinal cord significantly upregulated genes associated with neuroinflammation such as *Spp1*, *Ifitm3*, *Cd74*, and *Lgals3* compared to 6-week spinal cords after OxPC injection. Tissue sections were also examined by spatial RNAseq which showed aging associated transcriptional changes that were enriched in the OxPC lesion compared to the normal white or grey matter. Both single cell and spatial RNAseq revealed the upregulation of liver x receptor (LXR) activated genes by microglia in response to OxPCs and LXR activation by the agonist GW3965 attenuated OxPC mediated neurodegeneration in both 6-week and 52-week-old mice. Moreover, in vitro and in vivo experiments demonstrated altering the levels of the aging-upregulate gene *Spp1* (osteopontin) significantly impacted the severity of OxPC mediated neurodegeneration, at least in part by altering microglia/macrophage functions. Thus, aging is associated with significant transcriptomic changes by microglia and macrophages in the spinal cord, which were further altered after aging exacerbated OxPC mediated neurodegeneration. These findings also highlighted pathways associated with LXR activation and osteopontin signaling that may regulate neurodegeneration and MS progression with aging.

Disclosures: Y. Dong: None. **R.W. Jain:** None. **B.M. Lozinski:** None. **C. D'Mello:** None. **F. Visser:** None. **S. Ghorbani:** None. **V. Yong:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Grants Council of the Hong Kong SAR Grants 26104216
Research Grants Council of the Hong Kong SAR Grants 16101315
Research Grants Council of the Hong Kong SAR Grants16124916
Research Grants Council of the Hong Kong SAR GrantsC6009-17G
Research Grants Council of the Hong Kong SAR GrantsAoE/M-604/16

Title: Dna damage induced neuroinflammation in neurodegenerative disease

Authors: *F. SONG[1], J. MA[1], J. AW[2], D. C. LEUNG[3], K. HERRUP[1];
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Abstract: DNA damage induced neuroinflammation in neurodegenerative disease Fiona Song, Jack Ma, Jacqueline AW, Danny Leung, Karl Herrup
Cytoplasmic DNA (cytoDNA) is a strong immune stimulus that protects the cell against invasion by DNA viruses. Intriguingly, cytoDNA is also found in the absence of viral infection in conditions such as cancer, immune system disorders and neurodegeneration. As we showed previously, in mouse microglia, ATM deficiency and compromised DNA repair lead to a substantial accumulation of cytoDNA. This has pro-inflammatory consequences in microglia, where cytoDNA primes an antiviral response via the cGAS/STING pathway and activates the AIM2 containing inflammasome which cleaves and activates cytokine precursors such as pro-IL-1β. Together, these two responses create a pro-inflammatory response with neurotoxic consequences. We have extended these studies to learn the life history of microglial cytoDNA. We found that blocking nuclear export with Leptomycin B dramatically reduced cytoDNA demonstrating that it is actively exported from the nucleus by a CRM1-dependent mechanism. Deep sequencing of cytoDNA purified from ATM-deficient microglia highlighted two important features of its origins. First, mitochondrial DNA contributes less than 1% to the total amount of cytoDNA. Second, the cytoDNA exported from the nucleus comes predominantly from transcriptionally inactive, intergenic regions, in particular, AT-enriched repetitive elements at the nuclear periphery. RNA sequencing of ATM-deficient microglia revealed a significant upregulation in pathways involved in autophagy-lysosomal function, and the concomitant

downregulation of the c-MYC proto-oncogene. The c-MYC response is consistent with its role as a negative regulator of TFEB, a master regulator of lysosome biogenesis genes. We validated this connection by showing, both *in vivo* and *in vitro*, the decrease ofthat MYC and phospho-MYC decrease in *Atm*$^{-/-}$ mice brain. In microglia, ATM dysfunction induced the accumulation of cytoDNA which coupled was associated with the perinuclear aggregation of lysosomes as well as TFEB-mediated lysosomal biogenesis. In the aggregate, our findings suggest that with the inhibition of ATM the cellular balance of cytoDNA production and digestion is compromised triggering a complex response of the innate immune system. This identifies cytoDNA as a potential target for modulating the immune response that contributes to many neurodegenerative diseases.

Disclosures: F. Song: None. **J. Ma:** None. **J. Aw:** None. **D.C. Leung:** None. **K. Herrup:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01AG051812-05

Title: Surgery induces age-dependent but transient changes in the inflammatory transcriptome of microglia in mice

Authors: A. K. LEONARD[1], Z. YIN[2], S. SILVEIRA[2], C. CHENG[2], Z. XIE[4], D. CULLEY[5], O. BUTOVSKY[2,3], G. CROSBY[1];
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Abstract: Cognitive dysfunction is common after anesthesia and surgery in geriatric patients and is often attributed to systemic inflammation and CNS immune responses. Microglia, the immune cells of the brain, have been implicated based largely on immunohistochemical and morphological measures of activation, but one clinical study suggests microglia are hypoactive postoperatively. We hypothesized that surgery induces upregulation of proinflammatory mediators in microglia that is age-dependent and persistent. Old (20 mo; n = 8 M, 8 F) and young (3 mo; n = 9 M, 10 F) C57Bl/6J mice were anesthetized with isoflurane (1.2% in 30% O2, 70% N2), given bupivacaine (0.25%, s.c.) at the incision site and meloxicam (0.1 ml of 0.5 mg/ml, s.c.) for analgesia, and underwent a 15 min laparotomy with bowel manipulation. The

wound was closed with suture and mice remained under anesthesia for 2 h, then recovered for 4 h until sacrifice. Age-matched controls (9 M, 8 F old; 9 M, 10 F young) had no surgery and received carrier gas for 2 h but were otherwise treated identically. Additional old male mice (n = 9 surgery, 10 control) received the same treatment and recovered for 48 h before sacrifice. Plasma was collected at sacrifice for measurement of IL6, CCL2, IL1B, and TNFa by Luminex assay. We isolated microglia from hippocampus and hemisphere by FACS (Ly6C- CD11b+ Fcrls+), extracted RNA, and measured expression of *ApoE, Tgfbr1*, *Clec7a*, *Il6*, *Il1β*, and the housekeeping gene *Gapdh* via qPCR. C_t values were normalized to the young control mean ($2^{-\Delta\Delta Ct}$). Results were analyzed by 2-way ANOVA with an alpha of 0.05. At 6 h, plasma IL6 and CCL2, but not IL1b and TNFa, were elevated old but not young mice. Microglia from the hippocampus and hemisphere of old mice had higher *ApoE*, *Clec7a*, and *Il1β*, and lower *Tgfbr1* than those from young mice. Surgery increased microglial *Il6* and *Tgfbr1* for both age groups. There was an interaction of age and surgery only for *Clec7a*, a marker of phagocytic microglia; surgery decreased microglial *Clec7a* only in old mice. By 48 h after surgery, there were no differences in the expression profiles of microglia between control and surgery mice. Using validated molecular markers to isolate microglia, we confirmed age-differences in these cells as demonstrated previously. Our data indicate that abdominal surgery in mice may transiently decrease the phagocytic activity of microglia but does not produce sustained microglial-dependent neuroinflammation in the aged brain.

Disclosures: A.K. Leonard: None. **Z. Yin:** None. **S. Silveira:** None. **C. Cheng:** None. **Z. Xie:** None. **D. Culley:** None. **O. Butovsky:** None. **G. Crosby:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIGMS 1R01GM128008

Title: Imaging of microglial activation in mice with acetaminophen-induced acute liver injury: a dual tracer microPET evaluation of neuroinflammation

Authors: Q. ZENG[1], *J. CARRION[1], **S. PALANDIRA**[1], L. TURECKI[1], A. FALVEY[1], D. HERSCHBERG[1], K. J. TRACEY[1,2], *V. A. PAVLOV[1,2];
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Abstract: Acute liver injury that progresses into acute liver failure (ALF) is a life-threatening condition with increasing incidence and cost of care. Brain dysfunction and neurological

complications have also been documented in ALF and this *hepatic encephalopathy* is a central diagnostic criterion for ALF. Among other factors in the brain, neuroinflammation has been shown to significantly contribute to the development of hepatic encephalopathy. Non-invasive evaluation of neuroinflammation is important for strategizing new diagnostic and therapeutic approaches in ALF targeting the brain. Here we utilized Micro Positron Emission Tomography (microPET) with [^{18}F]-Fluoro-2-deoxy-2-D-glucose (^{18}FDG) and ^{11}C-Peripheral Benzodiazepine Receptor ([^{11}C]PBR28) to evaluate brain microglia activation (indicating neuroinflammation) during murine acetaminophen (APAP)-induced acute liver injury. Male C57Bl/6 mice (13-15-week-old) were fasted overnight and injected i.p. with APAP (600 mg/kg) or vehicle (saline). Imaging was performed at 24h and 48h after APAP (n=9) or vehicle (n=8) administration. [^{11}C]PBR28 (~0.5 mCi) was injected via the tail vein followed by 60-min dynamic imaging acquisition and 10-min transmission scan on a Siemens Inveon MicroPET. Subsequently, ^{18}FDG (0.5 mCi) was injected i.p. with a 10 mins static scan acquired following 40 mins uptake. Brain images were reconstructed using 3D-OSEM with attenuation correction and aligned into standard anatomical space using individual^{18}FDG-templates within Pixel-Wise Modeling Software (PMOD 4.0). Statistical analysis was done using Statistical Parametric Mapping. MicroPET analysis revealed no significant differences between the two groups of animals at 24h, although significantly elevated serum aspartate aminotransferase (AST) levels (P=0.0001) following administration of the same APAP dose vs vehicle confirmed the presence of liver injury. At 48h significantly increased [^{11}C]PBR28 binding (P<0.05 vs controls) was determined in the cortex (granular insular cortex), dorsal striatum (caudate putamen), and other brain areas. These brain alterations were associated with significantly increased serum AST levels (P=0.0001) at 48h. In conclusion, these results demonstrate the presence of microglial activation (neuroinflammation) using microPET at 48h after the onset of APAP-induced acute liver injury. These findings support further development of non-invasive PET-based evaluation of neuroinflammation in the context of hepatic encephalopathy in preclinical and clinical settings of acute liver injury and ALF. This study was funded in part by NIH/NIGMS.

Disclosures: Q. Zeng: None. **J. Carrion:** None. **S. Palandira:** None. **L. Turecki:** None. **A. Falvey:** None. **D. Herschberg:** None. **K.J. Tracey:** None. **V.A. Pavlov:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IRSC
he scottish rite charitable foundation of Canada

Title: Alteration of M1/M2 ratio of microglial cells from the developing brain exposed to perinatal injuries

Authors: *M. MENGUS[1,2], R. IMANE[1], S. TREMBLAY[1,2];
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Abstract: Background: Extreme preterm infants are exposed to multiple inflammatory stressors over their neonatal period including perinatal cerebellar hemorrhage (CBH) and postnatal infection, known as two major risk factors for neurodevelopmental impairments. Given microglia involvement in inflammatory functions across the central nervous system, they may play a central role in the pathogenesis of cerebellar injury in developing brains.
Methods: Conditional transgenic mice dependent on diphtheria toxin intracerebellar injection to deplete *CX3CR1*-positive cells (mononuclear phagocytes including microglial cells) were bred and exposed to CBH at P2 combined or not with early inflammation (LPS). Using this transgenic mouse model allows microglial cells depletion prior to cerebellar insult. Microglia phenotypic changes across time will be analyzed by flow cytometry.
Results: Our preliminary data showed that prior to insult at postnatal day 2, the predominant phenotype of activated microglial cells is M2 pro-repair (48,74% *n*=3-6) compare to M1 pro-inflammatory phenotypes (0,88%; *n*=3-6) analyzed by flow cytometry from whole brain tissue (*n*=8-9). Two weeks after being exposed to diverse perinatal insult combination (postnatal day 15), mouse pups showed a significant change of their M1/M2 ratio compare to controls. We observed an increase of M1 phenotype (7,51%; *n*=4-7) after LPS-exposure compare to controls (1,66%; *n*=4-6). We measured also a significant decrease of activated microglial cells expressing M2 phenotype from all insult groups: LPS (8,90%; *n*=4-7*;**P=0.0025*), CBH-LPS (12,53%, *n*=4-7,***P=0.0025*) and CBH alone (26,30%, *n*=2) compare to controls (35,04%, *n*=4-6).
Conclusions: Perinatal insult exposure lead to changes in M1/M2 ratio from microglial cells over time, which may translate to altered immune responses induced by future stressors exposure.

Disclosures: M. Mengus: None. **R. Imane:** None. **S. Tremblay:** None.

Poster

P304. Neuroinflammation: Microglia Biology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P304.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Brain & Behavior Research Foundation NARSAD Young Investigator Grant 28380 to C.E.G.

NIH Grant 5R01NS114130 to S.R.

Title: Transcriptional profile of microglia and neuroinflammatory mediators associated with depressive-like behavior in chronic epileptic rats

Authors: *C. ESPINOSA-GARCIA[1], A. ROJAS[1], G. N. NEIGH[3], L. B. WOOD[4], S. BITARAFAN[4], H. XIAO[2], S. RANGARAJU[2], R. DINGLEDINE[1];
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Abstract: Epilepsy is a chronic neurological disease characterized by recurrent seizures. Epileptic patients frequently develop psychiatric comorbidities such as depression. Depression and epilepsy are associated with elevated brain inflammation, including microglial activation and pro-inflammatory cytokine production. Specific cellular transcriptional and cytokine profiles could provide insight into inflammatory pathways involved in the pathogenesis of epilepsy and comorbid depression. To better understand how seizures cause cellular and molecular changes in the epileptic brain that may lead to depression, we examined transcriptional changes in microglia and inflammatory mediator expression from chronic epileptic rats. **Methods:** Status epilepticus (SE) was induced in adult male rats with pilocarpine (380 mg/kg, s.c.); non-seizure controls received saline. After 60 min, SE was interrupted with diazepam. Depressive-like behavior was assessed by the sucrose preference test (SPT) and the forced swim test (FST) each month after SE. At 6 months after SE, a 24 h-video was recorded to evaluate the intensity of epilepsy measured as the number of spontaneous seizures. Next, rats were deeply anesthetized and perfused with PBS, brains were removed, longitudinally bisected, one hemisphere was used for cellular and molecular analysis and the other for histology and immunohistochemistry. CD11b+ microglia were isolated from cortices using magnetic activated cell sorting (MACS). Microglial gene expression was analyzed with the nCounter Neuroinflammation Panel (nanoString). Neuroinflammatory mediators were measured in cortical homogenates using a multiplexed Luminex assay. Ventricle enlargement was quantified in Nissl-stained coronal sections using the NanoZoomer slide scanner. Iba1 and GFAP markers were used to assess glial activation. **Results:** Transcriptomic analysis revealed alterations in expression of several genes, upregulation of complement C3 was the most relevant change in microglia isolated from SE rats. The chemokine RANTES was upregulated in cortical homogenates from SE rats compared to controls. SE rats displayed a persistent lower sucrose consumption, indicative of anhedonia. No differences were observed in FST immobility time. Among the 16 pilocarpine-treated rats, at least ten had one or more spontaneous seizures. Several SE rats exhibited enlarged lateral ventricles, indicating severe neuronal loss. Iba1 was upregulated in hippocampal CA3. Our findings confirm activated microglia and neuroinflammation persist in the chronic phase of the disease, and suggest potential therapeutic targets for epilepsy and comorbid depression.

Disclosures: C. Espinosa-garcia: None. **A. Rojas:** None. **G.N. Neigh:** None. **L.B. Wood:** None. **S. Bitarafan:** None. **H. Xiao:** None. **S. Rangaraju:** None. **R. Dingledine:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.01

Topic: C.09.Stroke

Title: Decoding hand, wrist, and forearm movements using a wearable neural interface in chronic stroke survivors.

Authors: *E. MEYERS[1], D. GABRIELI[1], L. WENGERD[2], N. TACCA[1], M. DARROW[1], D. FRIEDENBERG[1];
[1]Battelle Mem. Inst., Columbus, OH; [2]Ohio State Univ., Columbus, OH

Abstract: Prosthetics and orthotics have been recognized for decades as a potential means to restore hand function and independence to individuals living with impairment due to stroke. However, current practices are insufficient, and the majority of stroke survivors are left with long-term dysfunction of the arm and hand. A promising method for controlling upper limb assistive devices is to infer movement intention from muscle activity using electromyography (EMG). Here, we present a wearable neural interface called the NeuroLife® Sleeve which is like a compression sleeve worn on the forearm with embedded electrodes to record high-resolution surface EMG. In this IRB-approved study, we recorded EMG activity using the sleeve in eight chronic stroke survivors (less than 6 months after stroke) with a range of upper extremity motor impairment (Upper Extremity Fugl-Meyer: 7-38). In the study sessions, participants followed along with cues on a computer monitor and attempted 12 hand, wrist, and forearm movements during three separate 2-hour sessions. We demonstrate decoding of 12 movements, including multiple functional grasps, with an overall accuracy of 82% in simulated real-time situations. Furthermore, we present feedback from stroke survivors who tested the system which highlights the user-centric design of the sleeve, including being simple to don and doff, comfortable, portable, and lightweight. Lastly, the sleeve is in a form factor such that it can be used in the home without an expert technician, can be donned by a user or caretaker, and can be worn comfortably for multiple hours. Taken together, the NeuroLife Sleeve represents a platform technology to record and decode forearm muscle activity for the eventual control of assistive devices in a form-factor designed to meet user needs. Disclaimer: This is an investigational device, it's not commercially available, and it has not been cleared or approved by the FDA. Results discussed are from research studies performed.

Disclosures: E. Meyers: None. **D. Gabrieli:** None. **L. Wengerd:** None. **N. Tacca:** None. **M. Darrow:** None. **D. Friedenberg:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.02

Topic: C.09.Stroke

Title: Global Network Structure Deficits Persist in Forearm Surface Electromyography Graphs of Chronic Stroke Survivors

Authors: *D. GABRIELI, E. MEYERS, M. DARROW, L. WENGERD, D. FRIEDENBERG; Battelle Mem. Inst., Columbus, OH

Abstract: Stroke is one of the leading causes of long-term disability and can result in lasting cognitive and motor impairment. Emerging technologies utilizing high-density surface electromyography (EMG) have shown great promise in non-invasively quantifying differences in neuro-muscular activation. However, these technologies have been limited by hardware ease-of-use and frameworks to assess the muscular coordination for functional motion. Here we utilize the Battelle Neurolife® Sleeve, a 160-electrode wearable garment, to assess the EMG activity of six chronic stroke participants with limited hand function (1-6 on Chedoke-McMaster Stroke Assessment hand subscale) while attempting functional movements of the hand, fingers, and wrist. We build movement specific, functional network graphs between EMG electrodes located across the forearm and assess the differences between stroke subjects and a set of able-body controls. We find that both global efficiency and local clustering metrics are altered in stroke subjects, compared to able body controls. Additionally, network metrics were indicative of the level of impairment, with greater deficits shown in subjects with moderate or severe hand impairment when compared to mild-impairment or able body subjects. Our research indicates that topological differences assessed through complex network theory can provide a novel, quantifiable assessment for neuro-muscular coordination deficits in subjects with chronic stroke.

Disclosures: D. Gabrieli: None. **E. Meyers:** None. **M. Darrow:** None. **L. Wengerd:** None. **D. Friedenberg:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.03

Topic: C.09.Stroke

Title: Battelle | Efficient Mapping of Motor Points Using a High-Density Electrode Array

Authors: *I. W. BAUMGART, C. F. DUNLAP, S. COLACHIS, IV, L. WENGERD, M. DARROW, E. MEYERS;
Battelle Mem. Inst., Ohio, OH

Abstract: Functional electrical stimulation (FES) has been shown to facilitate the recovery of motor function following spinal cord injury and stroke by promoting neuroplasticity and reducing spasticity. FES is standardly administered at motor points (MPs), which are points on the skin surface where the current applied to achieve muscle contraction is minimized. Although muscle contraction can be achieved by placing stimulating electrodes in many configurations over the muscle of interest, electrodes placed over MPs can decrease the current necessary to achieve contraction, decrease discomfort, and decrease fatigue. The location of MPs has been described in literature, but identification in the clinic can be difficult and time consuming, especially when trying to identify motor points for many functional movements. Here, we present a method for the efficient identification of motor points for multiple functional movements using machine learning techniques and Battelle's high-density electrode array sleeve. We first reduce the dimensionality of the problem by using "region of interest" electrodes constituting deformable clusters of electrodes in the high-density array. We then use machine learning techniques such as genetic algorithms and Bayesian optimization to identify and map MPs. We show that this method reduces the overall calibration time of multiple functional movements compared to trained manual calibration. We also evaluate the consistency of these calibrations between subjects and between sessions with the same subject. Finally, we discuss the challenges in using this automated approach in stroke populations.

Disclosures: I.W. Baumgart: A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **C.F. Dunlap:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **S. Colachis:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **L. Wengerd:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **M. Darrow:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **E. Meyers:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.04

Topic: C.09.Stroke

Title: Maximizing Hand Function of Chronic Stroke Survivors Using a Low Profile Functional Electrical Stimulation Sleeve

Authors: *L. WENGERD, E. MEYERS, M. DARROW, D. FRIEDENBERG;
Battelle Mem. Inst., Columbus, OH

Abstract: The inability to grasp and manipulate objects is a common outcome of stroke that results in devastating effects on functional independence and quality of life. Functional electrical stimulation (FES) has proven to be an effective rehabilitation tool to decrease motor impairment in adult stroke survivors, yet current systems are limited in usability and functionality. Here, we present the NeuroLife® Sleeve, a breakthrough technology that addresses the shortcomings of current FES systems to restore hand and forearm function to adult stroke survivors. NeuroLife is a low-profile orthosis, comparable to a compression sleeve, with 160 high-definition electrodes and a portable stimulator to stimulate forearm muscles and evoke finger, wrist, and hand movements during functional activities. In this IRB-approved research study, we used the sleeve to elicit various functional hand movements in three chronic stroke survivors (>6 months) with moderate-to-severe motor impairment (Upper Extremity Fugl-Meyer Assessment: 19-31) limiting their ability to complete functional tasks. Each participant wore the NeuroLife Sleeve to provide stimulation as they attempted various hand, wrist, and forearm movements. Participants showed a significant improvement in carrying out functional hand movements, as demonstrated by the ability to grasp and release objects and improvement in clinical assessment scores. One participant demonstrated a decrease in time completing a portion of the 9-Hole-Peg Test from 2 minutes and 42 seconds down to 19 seconds (an ~850% improvement), while another improved his score on the Action Research Arm Test (ARAT) by 6 points in one session (MCID: 5.7 points). Our research indicates the feasibility of the NeuroLife Sleeve as a rehabilitation tool or functional orthosis to maximize functional independence of adult stroke survivor with upper extremity motor impairment. © 2021 Battelle Memorial Institute. All rights reserved

Disclosures: **L. Wengerd:** A. Employment/Salary (full or part-time):; The Ohio State University. **E. Meyers:** None. **M. Darrow:** None. **D. Friedenberg:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.05

Topic: C.09.Stroke

Support: R01NS102917
K25HL140153

Title: Ultraflexible Neural-Thread enabled chronic quantification of neurovascular coupling in a mouse stroke model

Authors: *H. RATHORE[1,2,3], F. HE[1], B. NOBLE[2,1], L. LUAN[3,2,1];
[1]Rice Neuroengineering Initiative, Rice Univ., Houston, TX; [2]Applied Physics, Applied Physics Grad. Program, Smalley-Curl Institute, Rice Univ., Houston, TX; [3]Electrical and Computer Engin., Dept. of Electrical and Computer Engineering, Rice Univ., Houston, TX

Abstract: Neurovascular coupling (NVC), the close spatial and temporal relationship between neural activity and hemodynamics, has a central role of regulating the cerebral blood flow and maintaining normal brain function. However, the quantitative relationship between changes in hemodynamics and neural activity, particularly the spatiotemporal variations within the evoked brain region, remains murky. More importantly,although a broad spectrum of neurological and cerebrovascular diseases are known to affect neurovascular coupling, the changes In the coupling function and its longitudinal time evolution with the progression of the disorder remains largely unknown. Here, we integrate large-scale ultra-flexible Neural-Threads (NET) with functional optical imaging for simultaneous mapping of laminar neural activity at multiple locations, cerebral blood flow, and oxygenation. This novel multimodal neural platform enables quantification and spatiotemporal tracking of NVC over chronic periods. Using mouse models and whisker stimulation paradigms, we determine the cortical-depth specific NVC transfer function in normal brain states and how the functional form changes with time and location after artery-targeted strokes. The results reveal the pathological evolutions of neurovascular interaction after stroke and helps further our understanding on the interpretation of neuroimaging techniques that infer neural activity from hemodynamic responses.

Disclosures: H. Rathore: None. **F. He:** None. **B. Noble:** None. **L. Luan:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.06

Topic: C.09.Stroke

Support: National Institute On Disability, Independent Living, and Rehabilitation Research
National Institute of Health

Title: Immediate effect of stretching spastic elbow under Intelligent control for stroke survivors

Authors: *S. RAO[1], M. HUANG[1], S. G. CHUNG[2], *L.-Q. ZHANG[1];
[1]Univ. of Maryland, Baltimore, MD; [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Upper limb problems, including muscle spasticity, contracture, and weakness, are common after stroke. Elbow joint is one of the frequently and chronically affected structures with a manifestation of reduced range of motion (ROM) and weakness, which significantly impacts activities of daily living. Therefore, management of upper limb motor impairment has been identified as a top priority by stroke survivors, caregivers, and clinicians. The purpose of this study was to assess the immediate effects of strenuous dynamic stretching of elbow joint performed with help of an intelligent stretching device in chronic spastic stroke survivors. The intelligent stretching device could provide intensive and repetitive stretching to the spastic elbow

joint in sagittal plane (i.e., flexion and extension) to its extreme positions safely, with control of the stretching velocity and resistance torque to increase ROM, reduce spasticity and joint stiffness. Eight chronic stroke survivors (52.6±8.2 years old, 5 males/3 females) completed a single hourly intervention session. Elbow passive and active ROM, strength, passive stiffness (quantifying the non-reflex component of spasticity) and instrumented biceps tendon tapping test (quantifying the reflex component of the spasticity) were measured before- and after-stretching. Post stretching, there was a significant increase in active ROM of elbow flexion($p=0.021$) and extension ROM($p=0.026$). Also, elbow AROM and the spastic elbow flexors showed a trend of an increase in their strength. The intelligent stretching had a short-term positive influence on the passive movement ROM. Thereby may potentially be used to stretch spastic elbow joints repeatedly and regularly and subsequently help reduce upper limb impairments post stroke.

Disclosures: S. Rao: None. **M. Huang:** None. **S.G. Chung:** None. **L. Zhang:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.07

Topic: C.09.Stroke

Support: 1R01HD098073-01
18TPA34230052
R01HD092351

Title: Evaluation of objective methods for measuring ipsilateral motor potentials evoked by transcranial magnetic stimulation in stroke survivors with severe upper limb paresis

Authors: *A. MOHAN[1], X. LI[1], E. B. PLOW[1], J. S. KNUTSON[2], M. WIDINA[1], B. ZHANG[3], D. A. CUNNINGHAM[2];
[1]Biomed. Engin., Lerner Res. Inst., Cleveland, OH; [2]MetroHealth Rehabil. Inst., Cleveland, OH; [3]Case Western Reserve Univ., Cleveland, OH

Abstract: Introduction: Ipsilateral motor evoked potentials (iMEPs) elicited using transcranial magnetic stimulation (TMS) are often used to measure excitability of (uncrossed) ipsilateral pathways to paretic muscles. Evidence indicates ipsilateral pathways may serve as alternate resources to support recovery in stroke survivors lacking sufficient (crossed) ipsilesional substrate. However, there are no widely accepted methods for defining the presence or absence of iMEPs. Both manual and semi-automatic (objective) methods are used in the literature without clear, common guidelines. Here, we investigate the percent agreement of published objective methods against the manual method. A high agreement between the objective and manual methods would provide precision to the manual methods and could minimize the time and

human effort required for the analysis.Methods: Six chronic stroke survivors with severe upper limb hemiplegia (Upper Extremity Fugl-Meyer (UEFM) median = 18, range = 2.75), characterized by lack of 10-degree extension in wrist and fingers, underwent iMEP testing using TMS. IMEPs were collected from the paretic biceps brachii (BB) by delivering suprathreshold TMS (30-40 pulses) to contralesional motor areas. Participants were asked to maximally contract the paretic BB and the non-paretic triceps brachii simultaneously while turning their neck to the paretic side, a method that elicits more and larger iMEPs. Two experienced investigators subjectively measured the presence and absence of iMEPs (manual method), which involved identifying an MEP waveform in the ipsilateral biceps muscle 3-10ms after the appearance of an MEP waveform in the contralateral (non-paretic) biceps muscle. Seven objective methods based on standard deviation (SD) and mean consecutive differences (MCD) were used to measure the presence or absence of iMEPs. Reliability (percent agreement) of the objective methods was compared against the manual method.Results: Percent agreement of iMEP presence or absence between the two raters was 87.3%, indicating strong agreement. Of the objective methods, iMEP detection using criterion threshold of background mean electromyography (EMG) + 1SD and background mean EMG + 2SD yielded the best percent agreement with the manual method (73.3% and 70.9% respectively), indicating moderate agreement.Discussion: There is room for improvement for current objective methods to meet the percent agreement based on the manual method. Future work to improve the objective method (e.g., patient-specific feature detection and optimization) are being explored.

Disclosures: A. Mohan: None. **X. Li:** None. **E.B. Plow:** None. **J.S. Knutson:** None. **M. Widina:** None. **B. Zhang:** None. **D.A. Cunningham:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.08

Topic: C.09.Stroke

Support: NSF Grant 1934650
University of Delaware Research Foundation Seed Grant

Title: Determining the influence of movement speed and distance on the accuracy of upper limb proprioceptive estimates

Authors: *D. T. TULIMIERI, J. A. SEMRAU;
Kinesiology and Applied Physiol., Univ. of Delaware, Newark, DE

Abstract: Intact proprioception is required for accurate movement of the upper limbs. Compared to motor function, little is known about the effects of stroke on proprioception. This is because

current clinical assessments largely fail to directly assess proprioception. Recently, to mitigate this problem, robotic tools have been implemented to objectively measure proprioception. Previously, we have found that ~60% of individuals with stroke have impaired proprioception. Here, we build upon previous work to determine the influence of perceived movement characteristics on the estimation of proprioceptive information for upper limb movements. We hypothesized that proprioceptive accuracy will decrease as a function of parameter magnitude from a "typical" reaching movement, and that these effects will be amplified as a function of age and/or neurologic injury (i.e., stroke). Twenty-nine controls (N=20 young adults, N=9 older adults) and 3 stroke participants performed a bi-manual proprioceptive matching task. Here, the robot passively moved one of the participants arms (passive arm) and they were instructed to mirror-match the speed, length, and direction of movement with the opposite arm (active arm). Each robotic movement was defined by two parameters, speed and length. We assessed movements at 7 speeds (0.1-0.4m/s) and 5 distances (7.5-17.5cm). Participants completed 10 trials per combination, for a total of 350 trials. We determined proprioceptive accuracy using previously validated measures of position sense and kinesthesia including Initial Direction Error (IDE) and Peak Speed Ratio (PSR). IDE was defined as the angular deviation between the passive and active movements. PSR was defined as peak speed of the active arm divided by peak speed of the passive arm. Preliminary results demonstrated that proprioceptive errors significantly increased in older adults and stroke participants. Within groups, we found that these errors scaled as a function of movement speed and/or length for multiple parameters. We observed similar linear trends for the three groups, where IDE increased and PSR decreased with movement speed, and IDE decreased and PSR increased with movement distance. Notably, we observed that both age and stroke had an effect on the overall level of IDE and PLR accuracy, with stroke participants displaying the highest level of proprioceptive errors. Our results suggest that the characteristics of the reference movements used in a proprioceptive task (here, speed and distance) can influence the overall level of perceptual accuracy. Further, these difference in error are likely magnified in older individuals and individuals with stroke.

Disclosures: D.T. Tulimieri: None. **J.A. Semrau:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.09

Topic: C.09.Stroke

Support: NIH BRAIN Initiative Diversity Supplement UH3NS100541

Title: Stroke related changes in spinal reflex of multiple forearm muscles using high-density electromyography

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Abstract: Muscles work in coordination and are recruited differentially depending on the task being performed. These muscle activation patterns are thought to be encoded in the spinal cord. Stroke often produces clear changes in the function of spinal cord circuits, including reflexes, despite the injury being in the brain. Because the spinal cord is not directly injured by stroke, there is the possibility of some level of motor recovery by reversing the changes that occur in the spinal circuit. Therapy for motor control may be improved by having a better understanding of how the spinal circuit changes after neurological injury. We developed a non-invasive approach to test for stroke-induced changes in the spinal circuit by electrically stimulating peripheral nerves to measure reflex responses in forearm muscles. The electrical activity of activated muscles was measured by multiple high-density electromyography (HD-EMG) electrodes to capture the pattern of co-recruitment across multiple muscles. The subjects maintained hand postures used for functional tasks (i.e. power grip, hand opening) to facilitate spinal reflexes in muscles that are recruited preferentially for each task. The study was repeated for both forearms of able-bodied volunteers and stroke patients for comparison. We hypothesized that H-reflexes in extensor muscles will either be absent or the ratio of flexor/extensor H-reflex size will be increased significantly in the affected arm compared to the unaffected arm of stroke patients and both arms of able-bodied subjects during hand opening. We also hypothesized that larger ratios or absence of extensor reflexes are correlated with lower Fugl-Meyer scores and hand opening force. Finally, we hypothesized that H-reflexes in the flexor forearm muscles will be absent or significantly smaller in the affected arm compared to the unaffected arm of stroke patients and both arms of able-bodied subjects during power grip and that these results are negatively correlated with Fugl-Meyer scores. The results give us insight into the spinal circuit of stroke patients with motor impairment. Future work can investigate how to utilize spinal reflex recruitment patterns for personalized motor rehabilitation using electric stimulation targeting the spinal cord, peripheral nerves or muscles directly.

Disclosures: E. Bedoy: None. **K. Leitholf:** None. **E. Carranza:** None. **E. Guirola:** None. **N. Verma:** None. **I. Levy:** None. **G.F. Wittenberg:** None. **E. Pirondini:** None. **D.J. Weber:** None.

Poster

P331. Assessment of Stroke Damage

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P331.10

Topic: C.09.Stroke

Support: NSF Grant 1915083

Title: An Integrated Augmented Reality (AR) Encephalography (EEG) System for Detecting and Measuring Post-Stroke Spatial Neglect

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Abstract: Visual spatial neglect (SN) is a post-stroke syndrome that manifests as inattention to contralesional stimuli. Current clinical assessments such as the Behavioral Inattention Test (BIT) consists of simple pen-and-paper tests that do not adequately capture the extent or severity of SN, account for compensatory body movements, or yield consistent results. Our overall goal is to develop an objective method to detect and assess SN. To achieve this, we have developed a system that integrates augmented reality (AR) with electroencephalography (EEG) system for SN detection and measurement. In this setup, the displayed task follows the user's head movements and can be easily adaptable for rehabilitative purposes. We have implemented two computerized versions of the Starry Night Test for the Microsoft Hololens AR headset: a Clicker-Based Assessment to measure the ground-truth field-of-view (FOV) via reaction times to visual targets appearing among distractors, and an EEG-Based Assessment to evaluate the neural response to those targets using 14 electrodes. Five patients with SN and five without SN (WSN), identified using BIT scores (five male, five female, ages 27 to 80), performed the Clicker-Based Assessment followed by the EEG-Based Assessment. In this preliminary exploratory analysis, we first aimed to identify spectral features for optimal neglect detection. Reaction times from the clicker assessment were divided into fast and slow responses. A power ratio was calculated for five frequency bands (delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-45 Hz)) for each response as the power in the 400ms post-stimulus over that in the 200ms pre-stimulus. Two-way ANOVAs with Tukey HSD post-hoc tests of the power ratios of the fast responses at each electrode found statistically significant differences between SN and WSN groups primarily in the frontoparietal regions in the theta, alpha, and beta frequencies. Furthermore, a logistic regression analysis of the fast responses found that the right frontoparietal electrodes in the alpha band were most informative for separating SN from WSN. Similar analysis using the slow responses did not reveal significant results, suggesting that fast responses offer more information for class separation. Next, we estimated the FOV by classifying targets as fast or slow with common spatial pattern with linear discriminant analysis. For each participant, cross validation was conducted in estimating individual FOVs, resulting in high accuracies for all participants. These early results suggest that our system may be able to accurately detect and measure the extent of SN.

Disclosures: J. Mak: None. **D. Kocanaogullari:** None. **X. Huang:** None. **M. Shih:** None. **E.S. Grattan:** None. **E. Skidmore:** None. **G. Wittenberg:** None. **S. Ostadabbas:** None. **M. Akcakaya:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.01

Topic: D.03. Somatosensation – Pain

Support: NIH-NIBIB Nationala institute of biomedical imaging and engineering - Grant 5P41EB015896-19
NIH Grant 1R61AT009306-01
KIOM Grant KSN2021240

Title: Eeg hyperscanning to assess inter-brain connectivity in patient-clinician dyads during pain treatment.

Authors: *A. ANZOLIN[1,2], A. GRAHL[1,2], K. ISENBURG[3], M. BARTON-ZUCKERMAN[1,2], J. TOPPI[4], A. CIARAMIDARO[5], M. YUCEL[3], D.-M. ELLINGSEN[6], L. ASTOLFI[4], T. J. KAPTCHUK[7,2], V. NAPADOW[1,2];
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Abstract: Introduction. In chronic pain, a positive patient-clinician relationship is associated with higher patient satisfaction and treatment efficacy. In this study, we investigated brain-to-brain connectivity between patients and clinicians during an experimentally controlled augmented (empathetic) compared to limited (business-like) clinical interaction and assessed whether the context contributes to chronic pain relief and analgesia. **Methods.** We recorded simultaneous EEG (hyperscanning, 18 dyads, 64 channels each subject) from low back pain patients and acupuncturists during an evoked pain task with treatment and no-treatment trials. Brain data were reconstructed (algorithm eLORETA) on 17 Regions of Interest defined by our previous fMRI study (Ellingsen et al. 2020) and directed statistical connectivity networks were obtained by comparing Granger Causality-based estimates for treatment and no-treatment, separately for the augmented and limited group. **Results.** Patients in the augmented group, compared to the limited group, rated therapeutic alliance and clinician warmth significantly higher (Fig 1a). Pain intensity decreased after the treatment, irrespective of clinical context (Fig 1b). EEG analysis suggested that in Theta band (linked with empathy for pain in prior studies), inter-brain network density within social mirroring and pain/sensorimotor brain regions was altered when clinician treated evoked pain. The number of significant clinician-to-patient connections was higher in the no-treatment condition, while the number of patient-to-clinician connections were greater during treatment (Fig 1c). **Conclusion.** Both behavioral and brain-to-brain response across the patient/clinician dyad reflect clinical interaction context. Using an EEG hyperscanning approach we were able to identify inter-brain networks whose density and

directionality were modulated by the clinical context and by the presence of the pain treatment, highlighting new brain mechanisms underlying therapeutic alliance in chronic pain therapy.

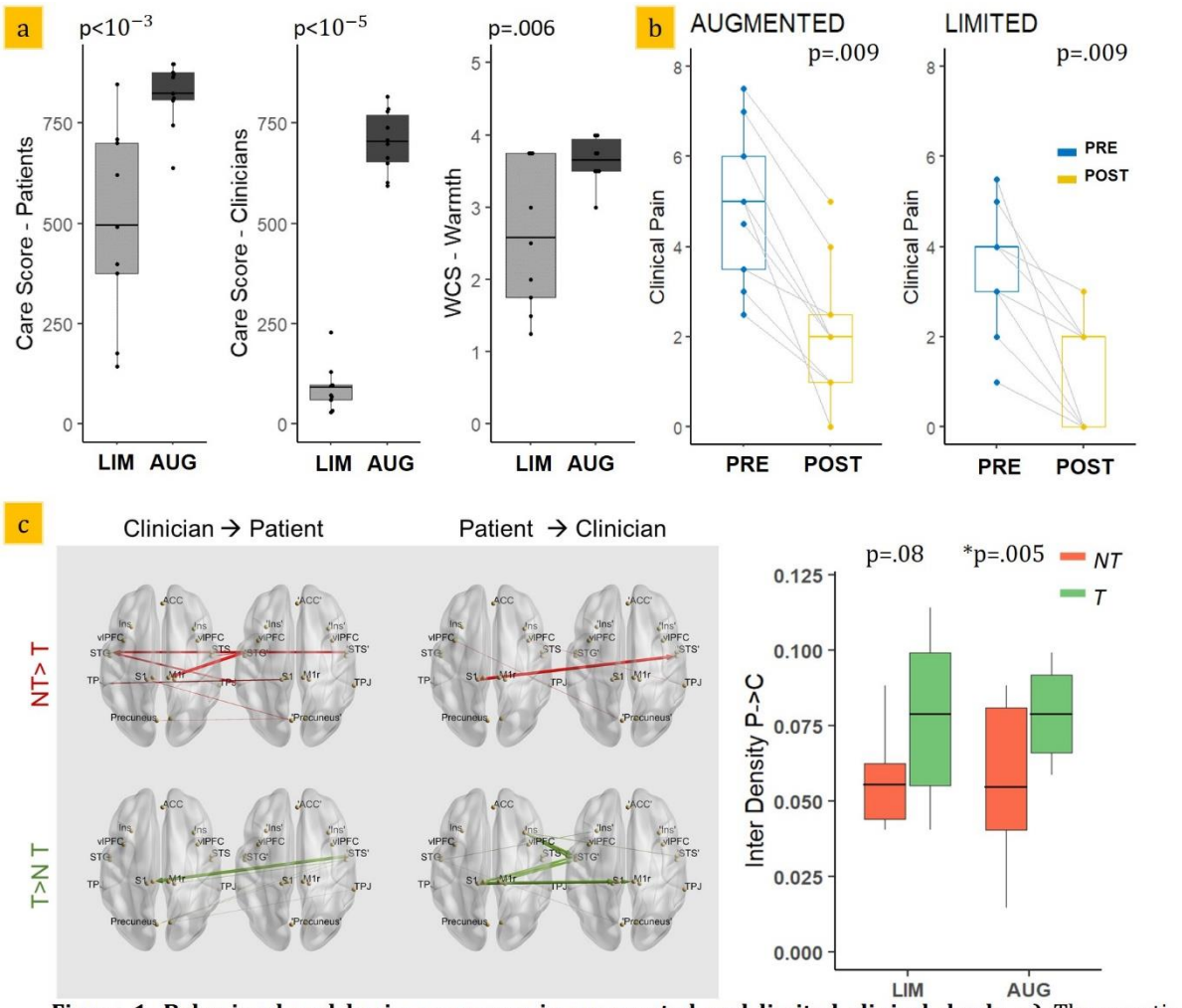


Figure 1. Behavioral and brain responses in augmented and limited clinical dyads. a) Therapeutic alliance (CARE score) and perceived warmth were rated as higher in the augmented group compared to the limited. **b)** Clinical pain assessed before (PRE) and after (POST) low back pain treatment significantly decreased regardless of the clinical context. **c)** Inter-brain network obtained from EEG data in Theta band. The number of connections directed from the clinician to the patient was higher when the clinician was forced to watch the patient in pain with no treatment (NT). In contrast, the number of inter-brain connections from patient to clinician was higher during the treatment trials (T).

Disclosures: A. Anzolin: None. **A. Grahl:** None. **K. Isenburg:** None. **M. Barton-Zuckerman:** None. **J. Toppi:** None. **A. Ciaramidaro:** None. **M. Yucel:** None. **D. Ellingsen:** None. **L. Astolfi:** None. **T.J. Kaptchuk:** None. **V. Napadow:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.02

Topic: D.03. Somatosensation – Pain

Support: Biogen Inc. USA
The authors would like to thank Energipole (M. Mallart), M.Villain and Société Française de Médecine Esthétique (M. Legrand) for unrestricted support for Research on Parkinson's disease.

Title: Nigral neuromelanin content in isolated rapid eye movement sleep behavior disorder and Parkinson's disease: a magnetic resonance imaging study using artificial intelligence

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Abstract: Isolated rapid eye movement (REM) sleep behavior disorder (iRBD) is a prodromal stage of Parkinsonism characterized by abnormal behaviors and loss of normal muscle atonia during REM sleep. Most iRBD subjects develop Parkinson's disease (PD) or dementia with Lewy bodies (DLB) with a median conversion time of 7 years. At PD onset, 30 to 60% of the dopaminergic neurons in the substantia nigra pars compacta (SNc) are already lost. These SNc neurons contain neuromelanin (NM) that can be detected using NM-sensitive magnetic resonance imaging (MRI).
In this study, we investigated the SNc NM neurodegeneration in iRBD, compared to PD patients and healthy volunteers (HV).
Participants were scanned at 3T MRI using 3D T1-w and NM images as part of the ICEBERG study (ClinicalTrials.gov: NCT02305147). Two experts performed SNc manual segmentations. Automatic SNc segmentations were performed using our proposed artificial intelligence (AI) framework, NigraNet that is a modified U-net architecture. NigraNet was trained on 54, validated on 6, and tested on the remaining 186 images. SNc volumes (Vol), corrected volume (C_{vol}=Vol/total intracranial volume) to normalize for the head size, signal-to-noise ratio, and contrast-to-noise ratio were obtained. A one-way general linear model analysis of variance adjusted for age and sex and post-hoc t-tests were performed. Receiver operating characteristic (ROC) analysis was computed for the diagnostic performance. Dice similarity coefficient was computed to compare the NigraNet and manual segmentations.
We analyzed 246 participants including 47 iRBD (MDS UPDRS OFF score: 11.7 ± 6.5), 144 PD (MDS UPDRS OFF score: 29.6 ± 7.9, disease duration: 1.5 ± 1.0 years) and 55 HV (MDS

UPDRS OFF score: 5.6 ± 5.3).We demonstrated that all the NM-based SNc measurements significantly differed between the three groups with C_{vol} of 0.16 ± 0.04 for iRBD, 0.14 ± 0.04 for PD, and 0.20 ± 0.04 for HV ($p<0.001$). We obtained significantly lower values in PD than in both iRBD and HV, and in iRBD than in HV except for SNR that did not differ between iRBD and HV. Using NigraNet, ROC analyses allowed differentiating iRBD and HV using C_{vol} with areas under the curve (AUC) of 0.79 and differentiating PD and HV using C_{vol} with AUC of 0.85. The SNc Manual segmentation results were also similar. There was high reproducibility with a DICE of 0.80 between NigraNet and manual SNc segmentations.
We demonstrated reduced neuromelanin content in iRBD with changes being at an intermediate level between values in HV and PD patients. Hence, NM MRI, coupled with our fast and user-independent NigraNet, has the potential to be the future biomarker for clinical trials of disease-modifying treatments.

Disclosures: R. Gaurav: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Grant funding from Biogen Inc. USA. **R. Valabrègue:** None. **N. Pyatigorskaya:** None. **G. Mangone:** None. **S. Leu-Semenescu:** None. **J. Corvol:** Other; Served in advisory boards for Air Liquide, Biogen Inc., Denali, Ever Pharma, Idorsia, Prevail Therapeutic, Theranexus, UCB; has received grants from Sanofi and the Michael J Fox Foundation. **M. Vidailhet:** None. **I. Arnulf:** Other; Received honoraria from Idorsia Pharma unrelated to this study. **S. Lehéricy:** Other; Received grant funding from Biogen Inc.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.03

Topic: D.03. Somatosensation – Pain

Support: NIH P01-AT009965
NIH R33-AT009306
NIH R01-AT007550
NIH R01-AR064367

Title: Linking day-to-day fluctuations in chronic pain to neural correlates of pain anticipation and clinical outcomes of cognitive behavioral therapy

Authors: *M. BARTON-ZUCKERMAN[1], A. GRAHL[1], A. LAZARIDOU[2], M. PASCHALI[2], J. LEE[1], M. BERRY[1], L. ISARO[2], A. ANZOLIN[1], K. ISENBURG[1], R. EDWARDS[2], V. NAPADOW[1];
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Abstract: Previous studies suggest that greater day-to-day fluctuations in clinical pain predict better outcomes following interventions. Specifically, patients with greater fluctuations in pain at baseline experience greater placebo effects. However, the neural correlates of this phenomenon are largely unknown. In this longitudinal neuroimaging study, we assessed the influence of daily reported pain variability on brain response to nociceptive stimuli and clinical outcomes following behavioral therapy. Female fibromyalgia (FM, N=78) patients used a diary to report average daily pain severity for seven days before and after an 8-week intervention of cognitive behavioral therapy (CBT, N=49) or a matched education control intervention (EDU, N=29). Pain fluctuation was calculated using the standard deviation of all diary entries completed before intervention for each subject. Before the intervention, patients also completed an evoked pressure pain paradigm during whole-brain fMRI (Siemens 3T MRI, TR/TE=1250/33ms, voxel size 2mm3 isotropic, SMS MB acc. factor 5). This included six cuff stimulations of either non- or moderately painful pressures (calibrated to a 0 or 40 out of 100 intensity on a visual analogue scale). Clinical outcomes included the Pain Catastrophizing Scale (PCS), a specific target of CBT. We found that greater day-to-day clinical pain fluctuations at baseline were associated with lower ratings of evoked pressure pain during the MRI ($r = -0.23$, $p = 0.049$). Additionally, greater day-to-day clinical pain fluctuations were also associated with greater post-therapy improvement in pain catastrophizing for the CBT group (Mean = -7.5, SD = 8.2, $r = -0.32$, $p = 0.02$) but not education control (Mean = -5.2, SD = 9.6, $r = 0.29$, $p = 0.17$). Furthermore, greater day-to-day clinical pain fluctuations were negatively correlated with BOLD fMRI responses during the anticipation period prior to evoked pressure in the CBT+EDU sample. Specifically, this association was seen in several nociception-processing areas (e.g., primary sensory cortex, insula, and cerebellum). Our results support previous literature which suggests that greater clinical pain fluctuations may boost clinical treatment effects by influencing expectations of pain. This highlights the potential predictive importance of assessing daily diaries for chronic pain characterization and suggests that greater fluctuations in pain may reflect a more malleable pain experience leading to optimistic expectations during pain processing and better clinical outcomes.

Disclosures: M. Barton-Zuckerman: None. **A. Grahl:** None. **A. Lazaridou:** None. **M. Paschali:** None. **J. Lee:** None. **M. Berry:** None. **L. Isaro:** None. **A. Anzolin:** None. **K. Isenburg:** None. **R. Edwards:** None. **V. Napadow:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.04

Topic: D.03. Somatosensation – Pain

Support: NCCIH R61-AT009306, R33-AT009306
NCRR P41RR14075, CRC 1 UL1 RR025758

MGH R90DA023427
NIH S10RR023401, S10RR019307, S10RR019254, S10RR023043
KIOM

Title: Fmri hyperscanning in chronic pain: neural and behavioral correlates of the patient-clinician relationship

Authors: *A. GRAHL[1], A. ANZOLIN[1], M. BARTON-ZUCKERMAN[1], K. ISENBURG[1], J. LEE[1], D.-M. ELLINGSEN[2,1], C. JUNG[1,3], J. GERBER[1], J. KELLEY[4,5], I. KIRSCH[5], T. KAPTCHUK[5], V. NAPADOW[1,6,7];
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Abstract: The quality of a clinical encounter is fundamental to shape patients' trust and build a collaborative relationship. Using synchronized real-time dual-brain approaches, insights into the patient's neural activity paired with the clinician's cognitive processes elucidate modulators of the patient-doctor relationship. Previous research suggests that a warm-empathic (*Augmented*) compared to a neutral, business-like (*Limited*) patient-clinician relationship can improve clinical outcomes. This longitudinal hyperscan fMRI study evaluates the influence of the clinical relationship on a course of therapy for chronic pain patients, and includes behavioral, neural, and clinical outcomes. FMRI hyperscanning and an evoked pressure pain-treatment-paradigm was applied. Seventeen women with fibromyalgia (mean age=41.41, SD=12.20), randomly assigned to either an *Augmented* (N=8) or a *Limited* (N=9) patient-clinician dyadic interaction style (trained acupuncturists), were scanned (2 synchronized Siemens 3T MRI scanners; TR/TE=1250/33ms; SMS MB acc. factor 5; voxel size $2mm^3$; 2x358 volumes) before and after 6 biweekly electro-acupuncture (EA) treatment sessions. Moderately painful cuff pressures, either treated or not treated with EA (8 trials each) by the acupuncturist, were applied to patients' left leg. Importantly, patients and clinicians were able to interact via a live video connection while being scanned simultaneously. The quality of the patient-clinician relationship and clinical outcomes were assessed (e.g. therapeutic alliance – THA, warmth – W, pain catastrophizing – PCS). As rated by patients, pooled therapeutic alliance and clinicians' warmth in the *Augmented* group ($mean_{THA}$=43.21, SD=2.40; $mean_{W}$=4.00, SD=0.0) were significantly higher than in the *Limited* group ($mean_{THA}$=22.79, SD=13.15; $mean_{W}$=2.54, SD=1.10; $t_{THA}(15)=4.31$, $p<0.001$; $t_{W}(15)=3.74$; $p=0.002$). PCS was significantly reduced after the intervention ($t(15)=3.79$; $p=0.002$) in the A*ugmented* (Δ-7.88) but not the *Limited* group (Δ1.44). Brain imaging analysis focused on social mirroring areas such as the temporo-parietal junction. Future analyses will link clinical outcomes and therapeutic alliance differences between the groups with brain and behavioral concordance metrics. Our preliminary results are highlighting the importance of investigating variable interaction quality during longitudinal clinical therapies and its influence on clinical outcomes and brain processing of nociceptive signaling. Hopefully, the results will inform future chronic pain treatment approaches how to harness the positive effects of the patient-clinician relationship.

Disclosures: A. Grahl: None. **A. Anzolin:** None. **M. Barton-Zuckerman:** None. **K. Isenburg:** None. **J. Lee:** None. **D. Ellingsen:** None. **C. Jung:** None. **J. Gerber:** None. **J. Kelley:** None. **I. Kirsch:** None. **T. Kaptchuk:** None. **V. Napadow:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.05

Topic: D.03. Somatosensation – Pain

Support: NIH F32 GM131479
NIH R35 NS097306
UCSF Program for Breakthrough Biomedical Research
Open Philanthropy Project

Title: Imaging Cortical Circuitry During General Anesthesia-Induced Analgesia

Authors: *J. A. P. WEINRICH[1], C. LIU[1], C. ANDOLINA[1], M. E. JEWELL[2], M. BERNSTEIN[1], A. I. BASBAUM[1];
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Abstract: INTRODUCTION: Many volatile general anesthetics (VGA) block the experience of pain by inducing unconsciousness, however, a select few can provide analgesia in conscious, responsive patients. Despite advances in our understanding of the effects of general anesthetics on regional measures of brain activity (fMRI, EEG), little is known about the mechanisms through which VGAs produce analgesia. In the present studies in the mouse, we investigated the influence of non-analgesic (isoflurane) and analgesic (nitrous oxide) anesthetics on the activity of neurons in the anterior cingulate cortex (ACC), a region that encodes affective/emotional features of the pain experience. Our objective is to uncover the neural circuit mechanisms through which general anesthetics alter the perception of pain. **METHODS:** In adult mice, we monitored the spontaneous activity of hundreds of individual ACC neurons before, during, and after the inhalation of isoflurane or nitrous oxide. To visualize neural activity, we used virally delivered genetically encoded fluorescent reporters of neural activity (GCaMP6f) expressed ubiquitously or within molecularly distinct neural subtypes. Changes in GCaMP6f fluorescence were captured via Inscopix miniscopes. **RESULTS:** Consistent with previous studies demonstrating that the global activity of the cerebral cortex is decreased during general anesthesia, we find that the rate of spontaneous neural activity decreases with increasing concentrations of isoflurane. Surprisingly, and in sharp contrast, during inhalation of nitrous oxide, we observed a significant increase in spontaneous activity of ACC neurons. Additionally, after dissecting the effects of general anesthetics on functionally distinct neurons within the ACC (i.e., excitatory vs subtypes of GABAergic inhibitory neurons), we demonstrate that inhibitory

interneurons exhibit increased susceptibility to isoflurane, while nitrous oxide preferentially increases activity in excitatory neurons. **CONCLUSIONS:** We conclude that non-analgesic and analgesic general anesthetics differentially alter neural activity in the ACC, a region of the brain that is a major contributor to the pain percept. In both patients and animals, decreases in ACC activity are generally associated with analgesia. Therefore, our finding that nitrous oxide, an analgesic, increased ACC activity, and that isoflurane, which is non-analgesic, decreases ACC activity, was unexpected. Our findings suggest that the analgesic potential of a general anesthetic results from the preferential modulation of the activity of particular subtypes of neurons rather than an overall reduction of neural activity.

Disclosures: J.A.P. Weinrich: None. **C. Liu:** None. **C. Andolina:** None. **M. Bernstein:** None. **A.I. Basbaum:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.07

Topic: D.03. Somatosensation – Pain

Support: NIH grant NS109287
NIH grant NS096246
NIH grant DK116624
NIH grant NS113189
NIH T32 Grant GM067795

Title: Stay or go? Neuronal activity in medial frontal cortex during a voluntary tactile preference task in head-fixed mice.

Authors: *A. KEYES, Y.-C. KIM, P. BOSCH, Y. USACHEV, G. ALDRIDGE;
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Abstract: The decision to move is influenced by sensory, attentional, and motivational cues. One such cue is the quality of the tactile input, with noxious or unpleasant sensations causing an animal to move away from the cue. Processing of painful and unpleasant sensation in the cortex involves multiple brain regions, although the specific role of the brain areas involved in voluntary, rather than reflexive movement away from unpleasant stimuli is not well understood. Here, we focused on the medial subdivision of secondary motor cortex, which is proposed to link sensory and contextual cues to motor action, and tested its role in controlling voluntary movement in the context of an aversive tactile cue. We designed a novel, 3D-printed tactile platform consisting of innocuous (grid) and mildly noxious (spiked) surfaces (50:50 % of total area), which enabled monitoring neuronal activity in the medial frontal cortex by two-photon

imaging during a sensory preference task in head-fixed mice. We found that freely moving mice spent significantly less time on a spiked-surface, and that this preference was eliminated by administration of a local anesthetic. At the neuronal level, individual neurons were differentially modulated specific to the tactile surface encountered. At the population level, the neuronal activity was analyzed in relation to the events where mice chose to “stop-on” or “go-from” a specific tactile surface and when they “switched” surfaces without stopping. Notably, each of these three scenarios showed population activity that differed significantly between the grid and spiked tactile surfaces. Collectively, these data provide evidence that tactile quality is encoded within medial frontal cortex. The task pioneered in this study provides a valuable tool to better evaluate mouse models of nociception and pain, using a voluntary task that allows simultaneous recording of preference and choice.

Disclosures: A. Keyes: None. **Y. Kim:** None. **P. Bosch:** None. **Y. Usachev:** None. **G. Aldridge:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.08

Topic: D.03. Somatosensation – Pain

Support: Startup funds Pr Tétreault
Pilot grant Quebec Pain Research Network
Pilot grant Quebec Bio-Imaging Network

Title: Evaluation of hippocampal subfields volume relationship to disability, catastrophizing and anxiety in chronic low back pain patients

Authors: *M. SEAN[1,2,4], G. LÉONARD[3,5], P. TÉTREAULT[1,2,4];
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Abstract: Chronic pain is an important public health problem, specifically chronic low back pain (CLBP). Limbic brain structures and more importantly the hippocampus is known to be involved in the transition from an acute to a chronic pain condition. Investigating hippocampal subfield properties and their relationship to pain, quality of life and comorbid conditions in CLBP could reveal the interplay between hippocampus function and behavioral phenotype. In this exploratory study, we aimed to use Magnetic Resonance Imaging (MRI) to establish baseline values of bilateral whole hippocampus and three of its subfields and their relationship to the impact of pain on daily activities (Pain Outcome Questionnaire - POQ), catastrophizing (Pain Catastrophizing

Scale - PCS) and anxiety (State and Trait Anxiety Inventory - STAI-S/T) in healthy subjects and individuals with CLBP. So far, 21 participants (10 healthy subjects and 11 CLBP subjects) were recruited to acquire brain structural data using T1 MRI and psychometrics data. MRI data were analyzed using the automated pipeline HIPS from volBrain (volbrain.upv.es) that produce volumetric values (raw and normalized to intracranial volume) of three hippocampus subfields: CA1-3, CA4-Dentate Gyrus and the subiculum. In healthy subjects, Spearman's correlation (adjusted for age and sex) showed a correlation between STAI-T score and bilateral subiculum fields normalized volume (right: r=0.78; p=0.02 and left r=0.84; p=0.009), but not in the CLBP subjects. No correlations were observed with the STAI-S in both groups. In the CLBP group, a correlation was observed between PCS score and right hippocampus normalized volume (r=0.82; p=0.007). Also, there was a correlation between PCS score and bilateral CA1-3 fields normalized volume (right: r=0.96; p<0 .001 and left: r=0.74; p=0.02). There was no correlation with either CA4-DG or subiculum fields. Finally, there was a correlation between the POQ score and the right CA1-3 field normalized volume (r=0.91; p<0.001). These results are supported by the literature where PCS was linked with hippocampus subfields impairments. Also, our results suggest that the correlation obtained with the right hippocampus was mainly driven by the CA1-3 field as no other subfields was correlated to PCS. To conclude, these results contribute to the refinement of our understanding of chronic low back pain phenotype and hippocampus abnormalities. Furthermore, we are currently collecting longitudinal data on these participants to evaluate stability of these findings.

Disclosures: M. Sean: None. **G. Léonard:** None. **P. Tétreault:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.09

Topic: D.03. Somatosensation – Pain

Title: Classification of trigeminal pain using artificial intelligence and multimodal brain imaging

Authors: *T. H. LATYPOV[1,4], M. C. SO[5], P. S.-P. HUNG[1,4], M. R. WALKER[4], S. TOHYAMA[6], F. RUDZICZ[2,7,8], M. HODAIE[1,4,3];
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Abstract: Hypothesis
Trigeminal neuralgia (TN) is a severe type of chronic facial pain characterized by frequent

electric shock-like pain episodes. TN is one type of pain syndrome, which at present is defined based on clinical grounds. MR imaging can provide *in vivo* insights into the pathophysiology of chronic facial pain disorders and can assist with diagnostics. A large amount of MRI data cannot be easily analyzed using conventional statistical methods but benefit modern artificial intelligence (AI) tools. We hypothesize that AI-driven analysis of MR imaging can define subtypes of facial pain with greater accuracy than current conventional methods. The main objective of this study is to create an AI model capable of defining types of facial pain based on MR imaging data.

Methods

T1 and Diffusion-Weighted Imaging (DWI) data from 435 chronic facial pain and 118 healthy subjects have been analyzed. Cortical thickness, surface area, and thalamic volume measures were extracted from T1 data using Freesurfer. DWI data distortion correction and estimation of Diffusion Tensor Imaging (DTI) scalar volumes had been performed in FSL. DTI Scalar volumes transformed to the MNI template space and regional intensity measures were extracted using the Johns Hopkins University White Matter Atlas. Processed data was used to train Random Forest (RF) and Logistic Regression (LR) models to distinguish between different types of chronic facial pain and healthy control data.

Results

Classical TN can be distinguished from healthy controls with accuracies up to 95% with area under the curve (AUC) ~ 0.93 and 0.99 for RF and LR, respectively. Classifying between trigeminal neuropathic pain vs. healthy controls is up to 91% accuracy, on average (AUC~0.92 and 0.97 for RF and LR respectively). Both classifiers identified sets of important grey and white matter-based predictive metrics which were significantly different across compared groups ($p<0.05$). These include changes in temporal gyri grey matter, diffusivity changes in fornix, cingulum, and corpus callosum, and volume reduction of the pulvinar nucleus. Classifying between Classical TN vs Trigeminal Neuropathic pain is relatively inaccurate (with the accuracy of 51% and AUC of 0.55); however, comparing the important features revealed two grey matter structures (ipsilateral insular and contralateral orbital cortex) with significant univariate differences ($p<0.05$).

Conclusions

Our findings represent the first instance of an AI-driven diagnosis of pain type, which opens the pathway for a greater level of objectivity for the assessment of pain conditions.

Disclosures: T.H. Latypov: None. **M.C. So:** None. **P.S. Hung:** None. **M.R. Walker:** None. **S. Tohyama:** None. **F. Rudzicz:** None. **M. Hodaie:** None.

Poster

P404. Pain Imaging and Perception III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P404.10

Topic: D.03. Somatosensation – Pain

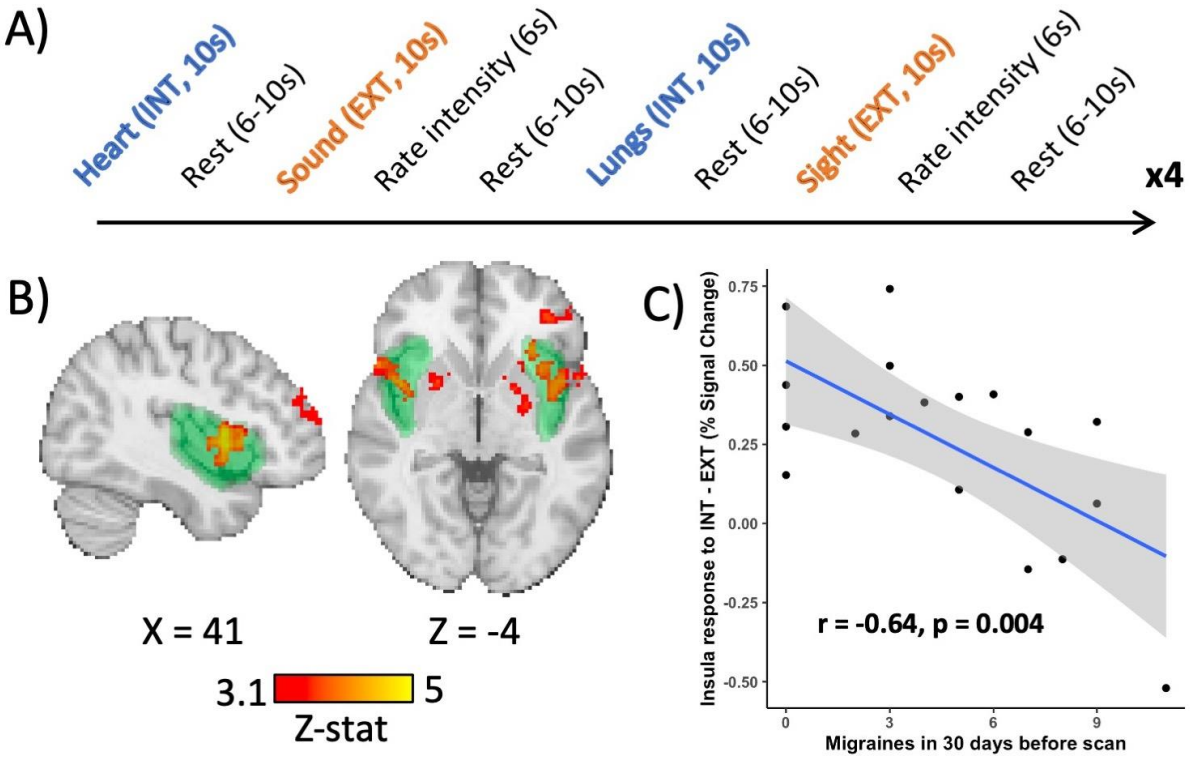
Support: NIH Grant P01 AT009965-01

Title: Insula response to interoception is inversely correlated with migraine frequency in patients with episodic migraine

Authors: *M. DATKO[1,2,3], Z. SCHUMAN-OLIVIER[1,2], L. BRUSAFERRI[1,3], H. HOUSMAN[3], S. TOHYAMA[1,3], K. ROUND[3], V. LE[3,5], M. HYMAN[3], H. CHENG[5,1], R. G. GARCIA[4,1,6], R. L. GOLLUB[6,1,3], R. EDWARDS[1,7], B. ROSEN[1,3], N. HADJIKHANI[1,3], M. LOGGIA[1,3], V. NAPADOW[1,3];
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Abstract: Background & Aims: Migraine is a highly prevalent chronic pain disorder. Interoception, the sensing of signals originating from within the body, is processed in insular cortex and may be altered in patients suffering from episodic migraine. The association between brain mechanisms of interoceptive awareness and clinical characteristics of migraine pathology has not been fully explored. We used functional MRI to explicitly investigate this association. **Methods**: 18 patients with episodic migraine (17 F, 1 M, mean age ± SD: 41.3 ± 13.1 yrs) were enrolled in an ongoing treatment study. All patients completed a novel interoceptive awareness task (**Figure 1A**) during BOLD fMRI acquisition in a 3T scanner (TR=2s, 3.125x3.125x3.6mm). During the task, patients were asked to focus their attention on and rate the intensity of sensation for two interoceptive conditions (visual cues: “heart” and “lungs”), as well as two exteroceptive conditions (“sight” and “sound”). Participants completed survey measures related to migraine symptoms, interoception, and trait mindfulness for at least 30 days prior to their scan. Participants also completed daily headache diaries to log migraine frequency, duration, and pain intensity. Bonferroni corrected Pearson correlations were calculated between insula BOLD response to interoception and each clinical and survey measure. **Results**: BOLD fMRI response for the interoception - exteroception task contrast included two clusters overlapping with mid-insula, bilaterally (**Figure 1B**). Significant inverse associations were observed between insula response and the Self-Compassion Scale ($r = -0.65$, $p = 0.003$), Freiburg Mindfulness ($r = -0.68$, $p = 0.002$), and number of migraine days in the 30 days prior to scan ($r = -0.64$, $p = 0.004$) (**Figure 1C**). **Conclusions**: Patients with episodic migraine showed robust insula response during a novel interoceptive awareness task, and this response was inversely correlated with recent migraine frequency, and measures of both trait mindfulness and self-compassion.

Figure 1. A) Task design including interoception conditions (visual cues for "Heart" and "Lungs") and matched exteroception conditions ("Sound" and "Sight").
B) Whole-brain results (n=18) from INT - EXT contrast ((Heart+Lungs)-(Sound+Sight)). BOLD response values used for correlations with clinical and survey measures were extracted from voxels of intersection between the whole-brain results (red-yellow) and the anatomical insula mask (green).
C) Correlation between BOLD response in insula cortex and number of migraines reported within 30 days prior to MRI scan.



Disclosures: M. Datko: None. **Z. Schuman-Olivier:** None. **L. Brusaferri:** None. **H. Housman:** None. **S. Tohyama:** None. **K. Round:** None. **V. Le:** None. **M. Hyman:** None. **H. Cheng:** None. **R.G. Garcia:** None. **R.L. Gollub:** None. **R. Edwards:** None. **B. Rosen:** None. **N. Hadjikhani:** None. **M. Loggia:** None. **V. Napadow:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.01

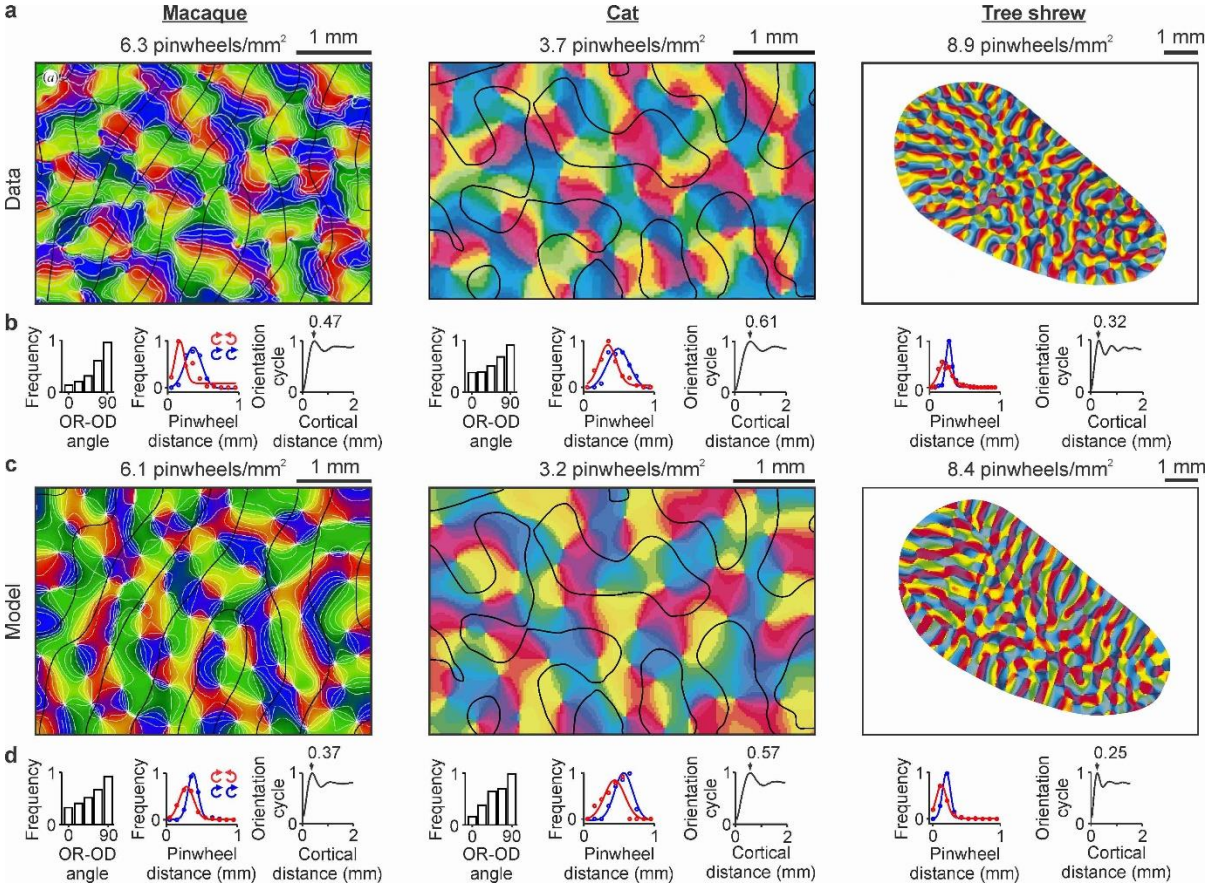
Topic: D.07. Vision

Support: EY05253
EY027361

Title: A theory of cortical map formation in the visual brain

Authors: *S. NAJAFIAN[1], E. KOCH[2], K. L. TEH[3], J. JIN[1], H. RAHIMI[1], Q. ZAIDI[1], J. KREMKOW[3], J. M. ALONSO[1];
[1]Suny Optometry, NYC, NY; [2]Biol. and Biol. Engin., Caltech, Pasadena, CA; [3]Neurosci. Res. Ctr., Charité-Universitätsmedizin Berlin, Berlin, Germany

Abstract: The cerebral cortex receives multiple afferents from the thalamus that become organized in topographic maps for different sensory modalities. Each sensory map represents the multiple dimensions of a stimulus in a complex pattern that varies across species for reasons unknown. Here we introduce a general theory of map formation that explains the natural diversity of visual cortical maps while replicating past and new measurements of multidimensional visual topography in different species. The theory develops maps through the sorting of thalamic inputs by type followed by synaptic adjustment through afferent co-activation and visual experience. Map diversity emerges from variations in the shape and size of the cortical domains representing the same stimulus position, which depend on the afferent sampling of sensory space (receptive-field tiling) and cortical space (axon-arbor tiling). We test the theory with a computational model that simulates visual maps and quantitatively compare them with experimental measurements. The model accurately simulates the orientation maps from three different species published in the scientific literature: macaque, cat, and tree-shrew (Figure 1). It reproduces the differences in orientation map geometry and pinwheel density across species (compare Figure 1a and c), the tendency for iso-orientation lines to run orthogonally to the ocular dominance borders in macaques and cats, the tendency of same-sign pinwheels to be farther apart than different-sign pinwheels, and the differences in average pinwheel distance and orientation periodicity across species (compare Figure 1b and d). The model also replicates the combined topography for multiple stimulus dimensions measured with multielectrodes in cat visual cortex for spatial position, ocular dominance, ON-OFF polarity, orientation preference/selectivity, homogeneity of orientation preference, spatial-frequency resolution and low-pass filtering. Because brain pathways use similar mechanisms for axon sorting and pruning, the theory may extend to other areas of the mammalian brain.



Disclosures: S. Najafian: None. **E. Koch:** None. **K.L. Teh:** None. **J. Jin:** None. **H. Rahimi:** None. **Q. Zaidi:** None. **J. Kremkow:** None. **J.M. Alonso:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.02

Topic: D.07. Vision

Support: MVLS DTP: 00544838
BBSRC: BB/S006605/1

Title: Biased distribution of fitted parameters in space reveals robust, yet spurious, angular tuning maps in the early visual cortex

Authors: *K. MORAVKOVA, J. FABIUS, A. FRACASSO;
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Abstract: Extending conventional modelling frameworks with the use of neurophysiologically-informed parameters is routinely performed in the field of computational neuroscience. This approach builds on the assumption that multitude of small-scale organisational principles of brain function can be recovered at coarse-scale if the employed measurement is sensitive enough to capture such features. While modelling single-unit receptive fields with the use of Gabor filter functions has uncovered some of the key organisational principles of neuronal tuning in the visual cortex, whether this benefit could be obtained at the scale of functional magnetic resonance imaging (fMRI) has not yet been explored via a forward-modelling framework. Here we extended the population receptive field (pRF) modelling approach, a method routinely used to characterise visually-evoked responses in humans, by implementing pRFs with Gabor filter configurations. Thus, we were able to estimate additional tuning properties (angle, phase, spatial frequency) at a single voxel level. The model revealed a coarse-scale organisation of angular tuning within the early visual cortex. This result was selective for Gabor filter pRF configurations with an asymmetric shape, a relationship which is in line with findings previously reported in monkey neurophysiology (see Figure 1). However, when we reapplied the model to a dataset of pRFs with uniformly randomly distributed configurations in space (i.e. lacking any coarse-scale pattern), we observed the same coarse-scale organisation, highlighting a non-neural origin of the observed result. This result is thus a cautionary case highlighting the potential caveats of extending the pRF modelling framework with assumptions grounded in neurophysiology. We believe these results underscore the continuing necessity to retest robust results with simulated datasets, irrespective of their consistency with theory.

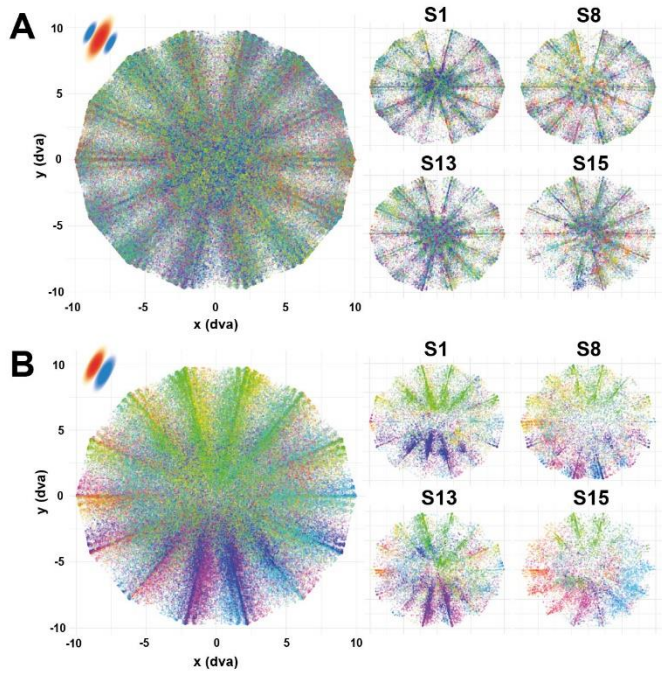


Figure 1: Coarse-scale angular tuning maps are dependent on the phase of the fitted Gabor filter pRF.

The plots show the results collapsed across all selected early visual areas (V1, V2, V3, V4, V3A, V3B). The colour represents the angular tuning of the Gabor filter pRF at the specified x and y coordinate. On the left, the plot shows data collapsed across all 18 participants. On the right, the single-participant-level angular tuning maps are shown for a subset of participants.

A: Data filtered to capture only Gabor filter pRFs with a symmetrical shape (defined by the phase parameter).

B: Data filtered to capture only Gabor filer pRFs with an asymmetrical shape (defined by the phase parameter).

The data shows a clear distinction between the coarse-scale angular tuning observed in space for the Gabor filter pRFs with symmetrical and asymmetrical shapes. While Gabor filter pRFs with symmetrical shapes show random distribution of angular tuning, the asymmetrical Gabor filter pRFs show spatially localised clusters of angular tuning, following the full range of 0-360 degree sensitivity across the visual cortex.

Disclosures: K. Moravkova: None. **J. Fabius:** None. **A. Fracasso:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.03

Topic: D.07. Vision

Title: A model for the development of binocular congruence in primary visual cortex

Authors: M. SOMARATNA, *A. W. FREEMAN;
Univ. of Sydney, Sydney, Australia

Abstract: INTRODUCTION. A recent study has provided insight into the development of binocular congruence. Chang et al. (*Neuron*, 107, 338, 2020) compared the interocular difference in preferred orientations of cortical neurons: the preferences differed substantially at the age of eye opening but matched well after visual experience. How does experience turn the monocular preferred orientations towards each other? We investigated this question by adding a second eye to an existing model (Freeman, *J. Neuroscience*, 41, 89, 2021). METHODS. The model has multiple on- and off-centre subcortical channels driven by each eye. All channels converge onto excitatory neurons in layer 4 of primary visual cortex. Development in the model occurred through Hebbian plasticity in the geniculocortical synapse and proceeded in two phases. In the first phase, which simulated the period before eye opening, stimulation of cortical neurons was assumed to be due to waves of retinal activity and was therefore monocular. The second phase simulated visual experience and the stimulus here was binocular. In the interests of physiological plausibility, the model was based on the known characteristics of the cat's X-cell pathway. RESULTS. Cortical responses before development were very weak because signals from neighbouring on- and off-channels cancelled each other when they converged at the cortex. Hebbian development increased response amplitude by spatially segregating on- and off-input. The segregation took the form of oriented bands and, because stimulation was monocular, the preferred orientation from stimulation via one eye was uncorrelated with that for the other eye. Binocular stimulation in the second phase of development remedied this mismatch. Where signals of one sign in one eye cancelled signals of the opposite sign at the same visual field location for the other eye, one eye was favoured and the fellow eye down-regulated. The result was an interocular match in orientation preference similar to that seen in empirical measurements. Further, the histogram of preferred binocular disparity across all neurons reflected empirical findings. Finally, a substantial fraction of cortical neurons in the fully developed cortex were dominated by a single eye: strong monocularity was seen in those neurons for which the monocular orientation preferences at the end of the first phase were close to orthogonal. This suggests that ocular dominance is a by-product of monocular stimulation before eye opening. CONCLUSIONS. Hebbian plasticity in geniculocortical synapses can explain the two-phase development of binocular congruence seen in the laboratory.

Disclosures: M. Somaratna: None. **A.W. Freeman:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.04

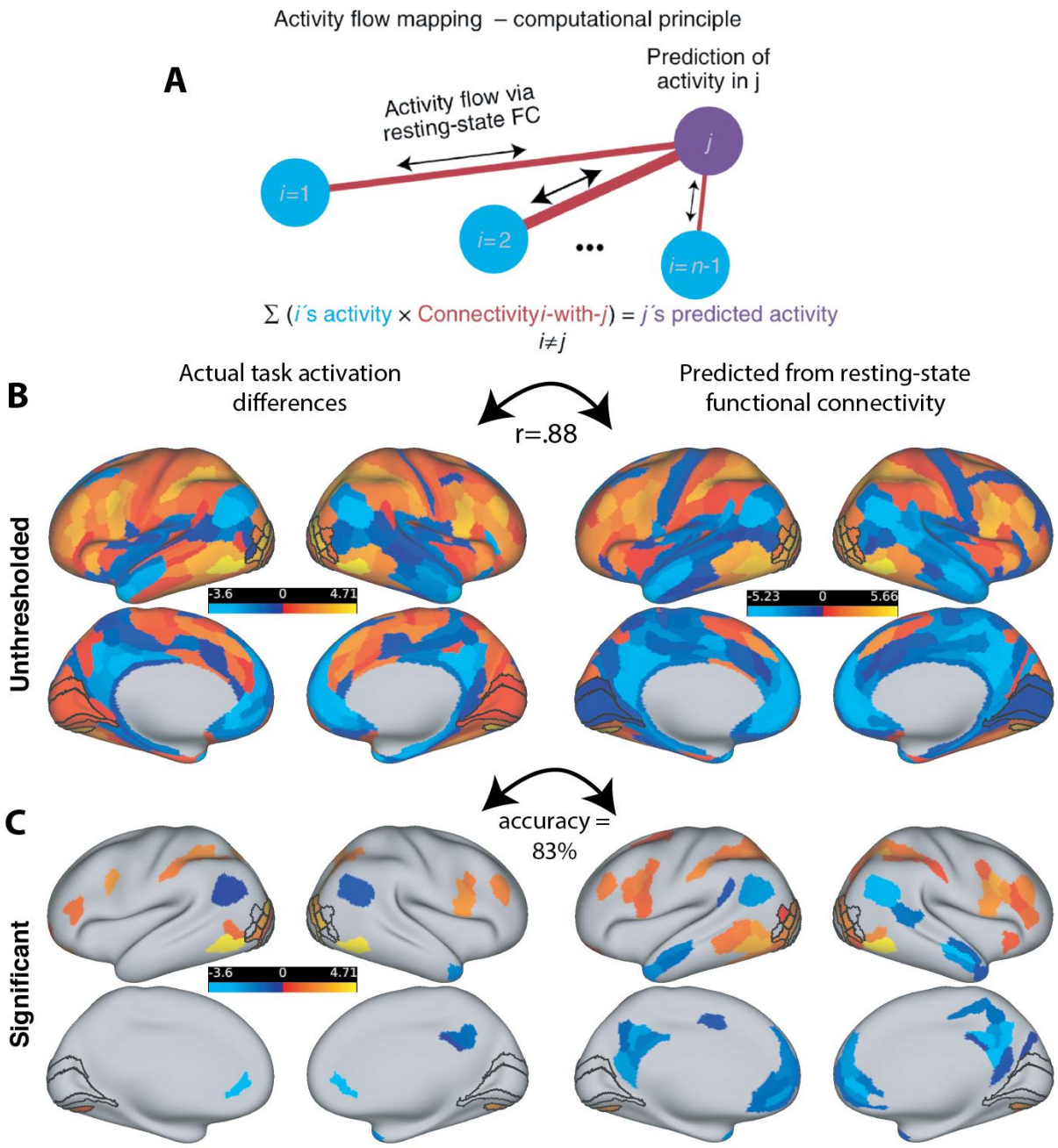
Topic: D.07. Vision

Support: K01MH108783

Title: Brain network mechanisms of visual shape completion

Authors: *B. P. KEANE[1], D. M. BARCH[3], R. D. MILL[4], S. M. SILVERSTEIN[2], B. KREKELBERG[4], M. W. COLE[4];
[1]Psychiatry, Neurosci., Brain/Cog. Sci., [2]Psychiatry, Neuroscience, Ophthalmology, Univ. of Rochester, Rochester, NY; [3]Dept Psychol & Psychiatry, Washington Univ., Saint Louis, MO; [4]Ctr. for Mol. & Behavioral Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Visual shape completion recovers object shape, size, and number from spatially segregated edges. Despite being extensively investigated, the process's underlying brain regions, networks, and functional connections are still not well understood. To shed light on the topic, we scanned (fMRI) healthy adults during rest and during a task in which they discriminated pac-man configurations that formed or failed to form completed shapes (illusory and fragmented condition, respectively). Task activation differences (illusory-fragmented), resting-state functional connectivity, and multivariate patterns were identified on the cortical surface using 360 predefined parcels and 12 functional networks composed of such parcels. Brain activity flow mapping (ActFlow) was used to evaluate the likely involvement of resting-state connections for shape completion. We identified 36 differentially-active parcels including a posterior temporal region, PH, whose activity was consistent across 95% of observers. Significant task regions primarily occupied the secondary visual network but also incorporated the frontoparietal, dorsal attention, default mode, and cingulo-opercular networks. Each parcel's task activation difference could be modeled via its resting-state connections with the remaining parcels ($r=.62$, $p<10^{-9}$; see Figure), suggesting that such connections undergird shape completion. Functional connections from the dorsal attention network were key in modeling task activation differences in the secondary visual network. Dorsal attention and frontoparietal connections could also model activations in the remaining networks. Taken together, these results suggest that shape completion relies upon a sparsely distributed but densely interconnected network coalition that is centered in the secondary visual network, coordinated by the dorsal attention network, and inclusive of at least three other networks. Additional neuroimaging results from schizophrenia and bipolar patients will also be considered using the methods described.



Disclosures: B.P. Keane: None. **D.M. Barch:** None. **R.D. Mill:** None. **S.M. Silverstein:** None. **B. Krekelberg:** None. **M.W. Cole:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.05

Topic: D.07. Vision

Support: NSF CRCNS IIS-1822683 (to K.K.)
NSF CRCNS IIS-1822929 (to T.N.)

Title: Using brain-optimized deep neural networks to investigate hierarchical computation in human visual areas V1-V4

Authors: G. ST-YVES[1], E. J. ALLEN[2], Y. WU[3], K. N. KAY[4], *T. NASELARIS[5,6];
[1]Neurosci., [2]Psychology, [3]Cognitive Sci., [4]Ctr. for Magnetic Resonance Res., Univ. of Minnesota Twin Cities Campus: Univ. of Minnesota Twin Cities, Minneapolis, MN; [5]Neurosci., Univ. of Minnesota, Minneapolis, MN; [6]Ctr. for Magnetic Resonance Res., Minneapolis, MN

Abstract: Visual areas V1, V2, V3, and V4 are often characterized as instantiating hierarchical computation. It has been hypothesized that the defining feature of this hierarchy is compositional dependence, i.e., each area applies a transformation to the outputs of the preceding area. Recently, task-optimized deep neural networks (DNNs) have been shown to yield impressively accurate predictions of brain activity in V1-V4, and for most networks, V1-V4 align with network layer depth. This result has been construed as evidence that V1-V4 are compositionally dependent. To carefully assess this interpretation, we analyzed the Natural Scenes Dataset, a massive dataset consisting of fMRI measurements of human brain activity (7T, 1.8-mm resolution) in response to up to 30,000 natural scene presentations per subject. We used this dataset to optimize DNNs to predict responses in V1-V4. In such brain-optimized networks, features distribute across layers in any way that improves the prediction of brain activity. This allows us to assess whether the alignment of V1-V4 with layer depth is a general property of DNNs that accurately predict brain activity. We found that when a single DNN is trained to jointly predict activity in V1-V4, alignment emerged in the sense that bottom layers of the DNN were more important for predicting activity in a given area than the areas above it in the hierarchy (e.g., bottom layers were more important for predicting activity in V1 than in V2-V4, and so on). Conversely, top layers were more important for predicting activity in a given area than the areas below it. Interestingly, when four distinct networks were independently trained to predict each of areas V1 through V4, there was no evidence for this type of alignment with layer depth. For example, the bottom layers in the network trained to predict V1 were less important than the bottom layers in the network trained to predict V4. Importantly, jointly and independently trained networks yield equivalent prediction accuracy. From these findings, we conclude that there is considerable flexibility in how to implement stimulus transformations that accurately predict brain activity in V1-V4. Some stimulus transformations are consistent with compositional dependence while others are not. We suspect that compositional dependence--without additional qualification--is too general a property to adequately explain the nature of the visual representations encoded in V1-V4.

Disclosures: G. St-Yves: None. **E.J. Allen:** None. **Y. Wu:** None. **K.N. Kay:** None. **T. Naselaris:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.06

Topic: D.07. Vision

Title: Interpreting models of neurons in visual area V4 via structural compression

Authors: *R. ABBASI-ASL[1], B. YU[2];
[1]UCSF, San Francisco, CA; [2]UC Berkeley, Berkeley, CA

Abstract: V4 is a large cortical area located between the primary visual cortex (V1) and higher visual areas in the inferior temporal lobe. Due to V4 neurons' highly non-linear responses, functions of neurons in V4 are less understood compared to other areas in the ventral visual pathway such as V1, V2, and IT. Recently, models based on convolutional neural networks (CNNs) have shown promise in predicting the activity of V4 neurons. More importunately, interpreting these CNN-based models has opened new doors to understand neural functions. However, these interpretations are based on models with hundreds of convolutional filters. Therefore, it is challenging to present a sparse set of filter bases for neurons via these models. To address this, we propose two algorithms to remove redundant filters in CNN-based models of V4 neurons. CAR compression prunes filters in a CNN based on their contributions to the image classification accuracy. Similarly, RAR prunes filters based on their contribution to the neural response prediction accuracy. Both CAR and RAR give rise to a new set of simpler accurate models for V4 neurons. These models achieve almost similar (for CAR) or higher (for RAR) accuracy compared to the original model. Using the compressed models, we are able to characterize each V4 neuron with a sparse set of filters. We show that V4 neurons are modeled via curvature or texture filters, as well as other more complicated filters. Our results present one of the first efforts to bridge between large-scale convolutional models of neurons and interpretable sparse networks.

Disclosures: R. Abbasi-Asl: None. **B. Yu:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.07

Topic: D.07. Vision

Support: Simons Foundation Grant SCGB-542965
NSF GRFP Grant
Mind, Brain, Computation and Technology Training Program

Title: Topographic deep neural networks predict the functional organization of the primate ventral visual pathway

Authors: *E. MARGALIT[1], H. LEE[4], J. J. DICARLO[4,5], K. GRILL-SPECTOR[2,6], D. L. K. YAMINS[2,6,3];
[1]Neurosciences Grad. Program, [2]Psychology, [3]Computer Sci., Stanford Univ., Stanford, CA; [4]Brain and Cognitive Sci., McGovern Inst. for Brain Res., Cambridge, MA; [5]Ctr. for Brains, Minds and Machines, MIT, Cambridge, MA; [6]Wu Tsai Neurosciences Inst., Stanford, CA

Abstract: The primate ventral visual pathway is organized into functional maps, including pinwheel-like arrangements of orientation-tuned neurons in primary visual cortex (V1) and patches of category-selective neurons in higher visual cortex. While deep convolutional neural networks (DCNNs) trained for object recognition accurately predict neural representations throughout the ventral pathway, they have no spatial layout for features at a given retinotopic location and are thus unable to predict the rich topographic organization of visual cortex. Here, we close this gap by first assigning each DCNN unit a position in a 2D cortical sheet, then training the network to minimize a cost function with two components: one encouraging accurate object recognition, and another favoring correlated responses among nearby units in each model layer (Figure 1A, 1B). We find that training with this composite spatial loss produces brain-like topographic maps in both early and later model layers (Figure 1B). Early layers contain smooth orientation preference maps with pinwheels, clusters of units preferring the same spatial frequency, and color-preference domains resembling V1 “blobs”. In a later layer of the same model, we observe clusters of category-selective units, e.g., face patches, whose spatial organization largely matches that found in primate higher visual cortex. Our model thus leverages local response correlations, which have been linked to theories of wire-length minimization, to accurately predict neuron responses and functional organization throughout the ventral visual pathway. In support of the wire-length minimization hypothesis, we find that our topographic DCNN would require shorter connections than a standard DCNN to support connections between similarly-tuned neurons within early (38% reduction) and later (31% reduction) model layers (Figure 1D). These results suggest that the functional organization of visual cortex can be explained by two constraints: the need to perform object recognition and pressure for local populations of neurons to have correlated responses.

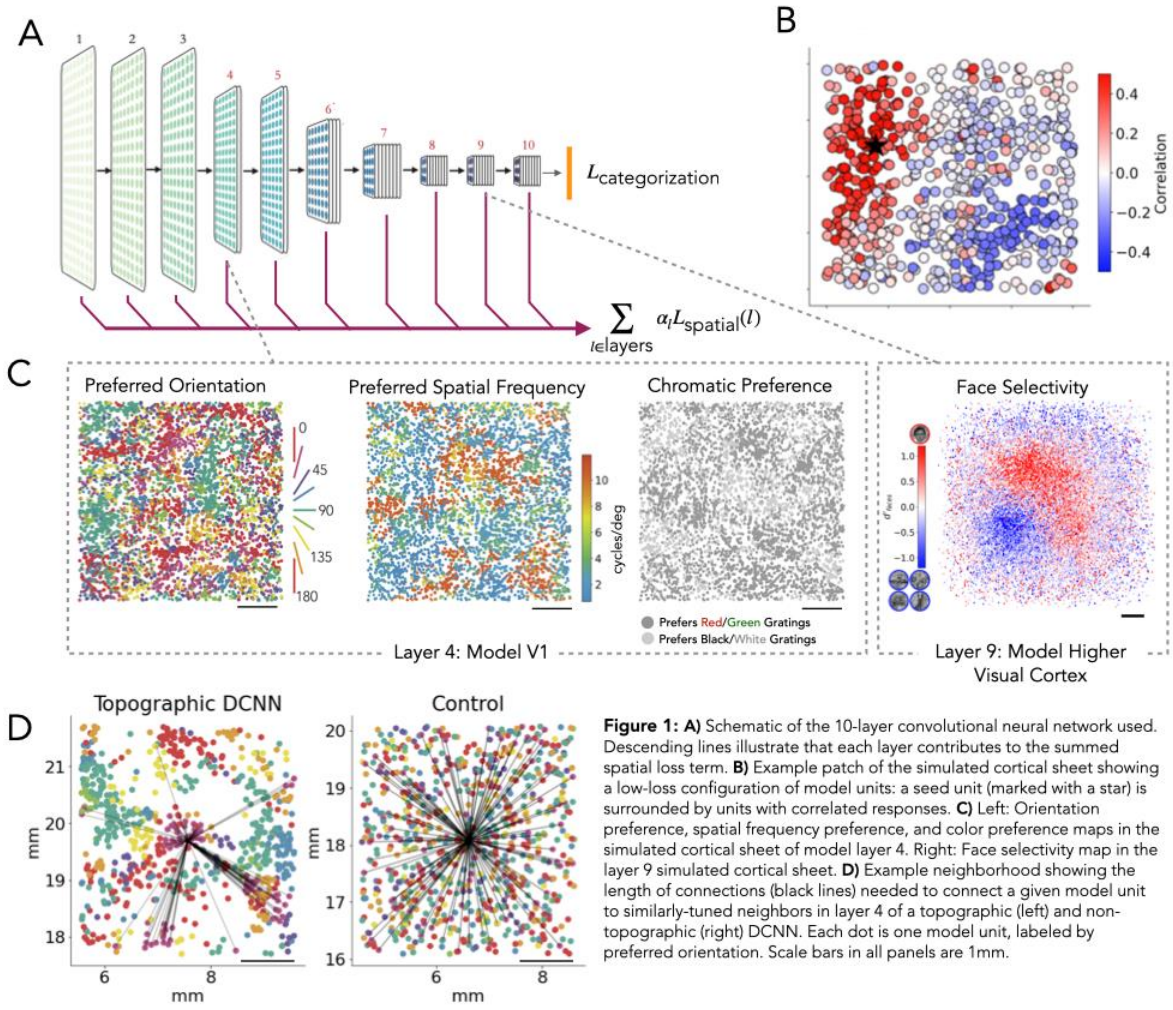


Figure 1: A) Schematic of the 10-layer convolutional neural network used. Descending lines illustrate that each layer contributes to the summed spatial loss term. **B)** Example patch of the simulated cortical sheet showing a low-loss configuration of model units: a seed unit (marked with a star) is surrounded by units with correlated responses. **C)** Left: Orientation preference, spatial frequency preference, and color preference maps in the simulated cortical sheet of model layer 4. Right: Face selectivity map in the layer 9 simulated cortical sheet. **D)** Example neighborhood showing the length of connections (black lines) needed to connect a given model unit to similarly-tuned neighbors in layer 4 of a topographic (left) and non-topographic (right) DCNN. Each dot is one model unit, labeled by preferred orientation. Scale bars in all panels are 1mm.

Disclosures: E. Margalit: None. **H. Lee:** None. **J.J. DiCarlo:** None. **K. Grill-Spector:** None. **D.L.K. Yamins:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.08

Topic: D.07. Vision

Support: NSERC
CFREF-funded VISTA Program

Title: Modularity and saccade influence in cortical vision networks: an fMRI and graph theory approach

Authors: *G. TOMOU[1], B.-R. BALTARETU[1], A. GHADERI[2], J. CRAWFORD[3];
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Abstract: Considerable regional evidence has accumulated for dorsal-ventral modularity in the visual system, but it is not clear how these modules function at the whole brain network level, or how these networks are influenced by naturally occurring saccades. We addressed these questions using graph theory analysis of fMRI data collected during a task where participants had to remember, then discriminate between two different object features. Seventeen participants judged whether a remembered object changed shape or orientation with or without an intervening saccade. BOLD activation from 50 cortical nodes was used to identify local and global network properties, which indicated greater inter-connectivity and efficiency of information transfer during saccades. A network modularity analysis revealed three sub-networks during fixation: a bilateral dorsal sub-network linking areas involved in visuospatial processing and two lateralized ventral sub-networks linking areas involved in object feature processing. Importantly, when horizontal saccades across the remembered object required visual comparisons between hemifields, the two lateralized ventral sub-networks became functionally integrated into a single bilateral sub-network. Comparisons of betweenness centrality between conditions identified several significant hub regions in occipital, parietal, and frontal cortex involved in linking distant network nodes during saccades. These results provide support of a ventral and dorsal stream distinction in human perception and show how hemispheric sub-networks are modified to functionally integrate information across saccades.

Disclosures: G. Tomou: None. **B. Baltaretu:** None. **A. Ghaderi:** None. **J. Crawford:** None.

Poster

P471. Function of Visual Circuits: Insights from Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P471.09

Topic: D.07. Vision

Support: HFSP postdoctoral fellowship LT000582/2019
JSPS grant 26290011
JSPS grant 17H06037
JSPS grant 372 C0219129

Fujitsu collaborative grant

Title: Sensory Plasticity Driven by Single Neurons in the Mouse Cortex

Authors: *R. AOKI[1], J. G. ORLANDI[2], A. BENUCCI[3];
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Abstract: Brain circuits acquire, implement, and update computations through the dynamics of recurrently connected neurons. The recurrent dynamics is enabled by the plasticity of the strength of these circuit connections, with diverse cellular and molecular mechanisms controlling the variability and changes in connectivity strength across neurons, as highlighted in several studies; however, the principles coordinating population-level changes for network computations remain largely elusive. Here, we found that optogenetic stimulation centered on a cortical cell—the *target* cell—could coordinate activity changes across hundreds of cells in the network in which it was embedded —the *surround*—and shifted the population encoding toward the feature preferentially encoded by the target cell. In particular, the repeated pairing of target cell (30 msec) -surround (10 msec, 1 msec delay, 5 pulses in 0.1 Hz) stimulation in the primary visual cortex of awake mice resulted in long-term, enhanced neuronal responsiveness to the stimulus feature—a specific orientation—preferred by the target cell. Moderately visually responsive cells showed the greatest magnitude of enhancement (0.05 ± 0.03, z-score, $n = 25$ experiments, $p < 0.002$, hierarchical bootstrapping), where the strongly visually responsive cells were mostly unperturbed ($p = 0.50$). Spontaneous responses were also affected, such that population activity became more aligned to the subspace defined by the visual modes (Explained variance: 19 ± 1 % for *before*, 21 ± 1 % for *after*, $p = 0.003$, $n = 25$), and cells similarly tuned to the target cell became more likely to participate in spontaneous neuronal assemblies ($p = 0.264$ for *before*, $p = 0.011$ for *after*). Our results reveal a surprising form of plasticity in adult cortical networks that is sensitive to the activation of targeted neurons and shows differential plasticity in weaker and stronger neural encoders, which highlights a mechanism that balances plasticity and stability of sparse feature representations. Our work also provides a proof of principle for technologies aiming to sculpt brain computations with spatially localized activations.

Disclosures: R. Aoki: None. **J.G. Orlandi:** None. **A. Benucci:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.01

Topic: E.03. Basal Ganglia

Support: NIMH R01-MH115030

Title: Functional diversity of frontostriatal circuits along the anterior-posterior striatal axis

Authors: *K. CHOI[1], E. PIASINI[2], L. VARGAS[3], E. DÍAZ-HERNÁNDEZ[3], M. SUBRAMANIYAN[1], M. V. FUCCILLO[1];
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Abstract: Frontostriatal circuits have been extensively implicated in the cognitive control of behavioral output for both social and appetitive rewards. The incredible diversity of prefrontal cortical function is strongly dependent on its synaptic target, with cognitive control of motor output mediated by connectivity to the dorsal striatum. Given evidence for a functional divergence along the anterior-posterior axis of the dorsomedial striatum, we investigated how distinct frontostriatal sub-circuits contribute to neural computations essential for action selection. Neural circuit tracing revealed largely segregated prefrontal populations defined by anterior/posterior dorsomedial striatal target. We probed the functional relevance of this organization via neural recordings and manipulations during a feedback-based choice task. In vivo calcium imaging revealed a largely non-overlapping distribution of task-relevant neural coding, with prelimbic-anterior dorsomedial striatum (PL-ADMS) circuits uniquely signaling action-related behavioral events and responses to negative outcomes while prelimbic-posterior dorsomedial striatum (PL-PDMS) neurons encoded internal representations of value and integrated choice-positive outcome signals. Consistent with this divergent prelimbic neural coding based on striatal target, optogenetic inhibition of PL-ADMS strongly impacted behavioral control in response to negative outcomes while PL-PDMS signals impaired task engagement and strategies following positive outcomes. Together these data suggest a distributed model of action control for prefrontal neurons defined by A/P striatal target.

Disclosures: K. Choi: None. **E. Piasini:** None. **L. Vargas:** None. **E. Díaz-Hernández:** None. **M. Subramaniyan:** None. **M.V. Fuccillo:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.02

Topic: E.03. Basal Ganglia

Support: NIH Grant KL2TR002554
Dystonia Medical Research Foundation Clinical Fellowship Training Program
Doris Duke Charitable Foundation FRCS
Dystonia Coalition Career Development Award

Title: Data driven linkage between network connectivity and dystonic behavior in writer's cramp dystonia

Authors: *N. BUKHARI-PARLAKTURK[1], M. FEI[2], M. DEREZINSKI-CHOO[2], J. VOYVODIC[2], S. DAVIS[2], A. MICHAEL[2];
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Abstract: Background: Dystonia is a network level brain disorder with limited understanding of interactions between functional networks. Prior studies have used a data driven approach to demonstrate abnormalities both between and within functional networks. However, these studies were focused on the "off" or resting disease state of task-specific dystonia and did not study functional networks when engaged in clinically relevant dystonic disease state. As a result, it remains unclear if the abnormal networks in the "off" disease state are also abnormal during the "on" disease state of task-specific dystonia. Furthermore, no study to date has demonstrated how changes in these functional networks correlate with manifestation of dystonic behavior. Methods: 12WC and 12 age-matched HV performed 3 motor tasks in fMRI: writing, sequence tapping and finger flexion-extension. Group independent component analysis was used to identify 11 functional networks. The functional networks were then correlated with the time course of the 3 motor tasks to identify highly correlated networks and determine group differences in network activation patterns. Differences in functional network connectivity was then evaluated between the two groups. Lastly, functional network connectivity was correlated with behavioral measures of dystonia.Results: Our analysis showed decreased functional connectivity between the basal ganglia and left sensorimotor network during the on-dystonia disease state. Behavioral analysis demonstrates positive correlation between writing legibility and functional connectivity of the cerebellum to basal ganglia and negative correlation of writing legibility and functional connectivity of the cerebellum to the orbitofrontal network. We also demonstrate a significant group difference in recruitment of the right sensorimotor network during dystonic behavior. Conclusions: Our analysis supports a global network disorder in which the basal ganglia and cerebellum play key roles. Based on our findings, we also propose a unifying mechanism of network disorders that may provide insight on primary disease and compensatory network changes. Our study is the first to link network changes during task performance to behavioral manifestations of a task-specific dystonia.

Disclosures: N. Bukhari-parlakturk: None. **M. Fei:** None. **M. Derezinski-Choo:** None. **J. Voyvodic:** None. **S. Davis:** None. **A. Michael:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.03

Topic: E.03. Basal Ganglia

Support: BBRF Young Investigator Grant PG010635

NINDS K99 award 1K99NS119788
Parkinson's Foundation Summer Student Fellowship
U19 Brain Initiative 5U19NS104649

Title: Direct projections from the external globus pallidus to thalamus and brainstem differentially impact behavior

Authors: *Z. GU[1], J. TANG[1], S. ROSENBERG[1], A. CHAKRAVARTHY[1], V. PAIXAO[2], L. HAMMOND[1], D. PETERKA[1], R. COSTA[1];
[1]Zuckerman Mind Brain Behavior Institute, Columbia Univ., New York, NY; [2]Champalimaud Neurosci. Program, Champalimaud Fndn., Lisbon, Portugal

Abstract: The external globus pallidus (GPe) is a key component of the basal ganglia system, yet its neuronal heterogeneity and contribution to action control have remained elusive. In the textbook model of basal ganglia function, the GPe comprises a single, homogenous population of neurons that serves as a simple relay in the striatopallidal pathway to inhibit movements. Using a combination of anterograde and retrograde tracings, we first found that GPe neurons are highly heterogenous: different subsets of GPe have distinct projection patterns, including previously uncharacterized subpopulations that project to the thalamus and brainstem. Second, axon-specific calcium imaging demonstrated that the activity of parafascicular thalamic nucleus-projecting (GPe→Pf) and pedunculopontine nucleus-projecting (GPe→PPN) GPe neurons are differentially modulated, such that GPe→Pf is strongly modulated during the performance of a learned lever pressing task while GPe→PPN is more modulated during locomotion. Third, optogenetic activation of GPe→PPN projection impeded locomotion, while manipulation of GPe→Pf had a strong effect on the execution of lever press. Therefore, our study provides anatomical, physiological, and functional evidence to support that GPe is an output nucleus of the basal ganglia, questioning the classical prokinetic versus antikinetic dichotomy of direct and indirect pathways. Our data strongly suggest a new model of basal ganglia function, in which striatopallidal pathway can either promote (via the novel GPe projections to thalamus and midbrain) or inhibit (via the classical subthalamic nucleus/STN to internal segment of the globus pallidus/GPi and the substantia nigra pars reticulata/SNr) movements depending upon the output channel(s). Supporting this notion, we further demonstrated that optogenetic activation of GPe→PPN projection blocks locomotion evoked by striatopallidal pathway stimulation in an open field exploratory task. Together, our data suggest that the striatopallidal pathway can function as direct output pathways via GPe projections to brainstem and thalamus to facilitate locomotion and learned action respectively.

Disclosures: Z. Gu: None. **J. Tang:** None. **S. Rosenberg:** None. **A. Chakravarthy:** None. **V. Paixao:** None. **L. Hammond:** None. **D. Peterka:** None. **R. Costa:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.04

Topic: E.03. Basal Ganglia

Support: GRANT VIEP-BUAP 2019-2020 awarded to Daniel Limon

Title: Effect of GPR55 inhibition into the external globus pallidus on fine motor skills in hemiparkinsonian rats

Authors: *F. PATRICIO MARTÍNEZ[1], E. MORALES-DÁVILA[3], A. PATRICIO[5], I. MARTINEZ-GARCIA[4], J. AGUILERA[6], J. PEREZ-AGUILAR[2], I. D. LIMON PEREZ DE LEON[7];
[1]Lab. of Neuropharm., [2]Facultad de Ciencias Quimicas, Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [3]Facultad de Ciencias Quimicas, [4]Lab. of Neurochemistry, Benemerita Univ. Autónoma de Puebla, Puebla, Mexico; [5]Lab. de Neurofarmacología-FCQ, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [6]Inst. de Neurociències, Univ. Autònoma de Barcelona, Cerdanyola del Vallès (Barcelona), Spain; [7]Benemerita Univ. Autonoma De Puebla FCQ Lab. Neurofarma, Puebla, Mexico

Abstract: Previous reports have shown that both cannabidiol (CBD) and CID16020040 blocks the GPR55-mediated effects of LPI. In addition, the expression of GPR55 mRNA in the basal ganglia (BG) has been described by *in situ* hybridization, especially in the striatum and external *globus pallidus* (GPe). These nuclei form the indirect pathway of BG are overactived in animal models of Parkinson's disease, leading to inhibition of GPe neurons and hypokinetic states. Because of this, the aim of the present work was to study the effect of GPR55 receptor inhibition in the GPe on fine motor skills of hemiparkinsonian rats in free movement. Male Wistar rats were used weighing 250-300 g to which 6-hydroxydopamine (6-OHDA) was administered in the nigrostriatal pathway [16 µg / 2 µL] by stereotaxic surgery. The staircase test was used to determinate the motor asymmetry of the experimental subjects in the 15th -19th days post-surgery and, later, at 20th days, a guide cannula was placed in the GPe to directly administer CBD [10 µM], CID160 [10 µM] and, LPI [10 µM]. Once the cannula was introduced, the drugs were administered at 28th, 29th, and 30th days and fine motor skills were assessed in staircase model. The results obtained show that the rats lessoned with 6-OHDA plus the intrapallidal injection with CBD or CID160 achieved a significant greater increase in the expert use of the forelimb contralateral to the lesion with 6-OHDA, but not with intrapallidal administration with LPI. While, an improvement was observed in the experimental groups that were administered CBD and CID160 of rats injured with 6-OHDA on fine motor skills: pronation, grasp and supination. In summary, our results suggest that the inhibitory effects of CBD and CID160 on the GPR55 receptor in the GPe of hemiparkinsonian rats reverse motor impairment possibly by regulating GABAergic neuronal activity.

Disclosures: F. Patricio Martínez: None. **E. Morales-Dávila:** None. **A. Patricio:** None. **I. Martinez-Garcia:** None. **J. Aguilera:** None. **J. Perez-Aguilar:** None. **I.D. Limon Perez De Leon:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.05

Topic: E.03. Basal Ganglia

Support: NIMH R03 NS096494
NIMH R01 MH093672
SNSF #P2EZP3_168841
SNSF #P400PB_180841

Title: The basal ganglia direct pathway supports motor control via GPe axon collaterals

Authors: *M. LABOUESSE[1], A. TORRES-HERRAEZ[1], J. VILLARIN[1], J. GREENWALD[1], X. SUN[1], A. TANG[1], M. ZAHRAN[1], S. LAM[2], J. BONAVENTURA[2], F. DE CARVALHO[1], C. LACEFIELD[1], M. MICHAELIDES[2], S. CHAN[3], O. YIZHAR[4], C. KELLENDONK[1];
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Abstract: In the classical model of the basal ganglia (BG) two segregated and functionally opposing pathways connect its input, the striatum with its midbrain outputs the substantia nigra reticulata (SNr) and entopenduncular nucleus (EP). Direct pathway spiny projection neurons (dSPNs) project to the SNr and EP while indirect pathway iSPNs project to the GPe. However, many recent studies have challenged both the functional dichotomy and the anatomical organization delineated by the classical BG model. For instance, all major BG nuclei send at least a subset of arborized axons collateralizing into one to four BG target regions. These axon collaterals could help shape BG information flow in space or time by sending copies of the same signals to distinct brain regions. Axonal “bridging” collaterals arising from dSPNs are a prominent example: axonal fibers arising from SNr-projecting tracts, yet targeting the GPe, the classical projection area of iSPNs. They generate half the density of GPe terminals produced by iSPNs, but their significance for behavior is still unknown. Here, we use *in vivo* optical and chemogenetic tools combined with deep learning approaches to dissect the roles of bridging collaterals in motor function. We found that dSPNs projecting to the SNr send synchronous motor-related information to the GPe via axon collaterals. We then found that inhibition of native activity in dSPN GPe terminals impaired motor activity and function via regulation of pallidostriatal Npas-1 neurons. We propose a model by which dSPN GPe collaterals act in concert with the canonical terminals in the SNr to support motor control by inhibiting Npas1 signals going back to the striatum.

Disclosures: M. Labouesse: None. **A. Torres-Herraez:** None. **J. Villarin:** None. **J. Greenwald:** None. **X. Sun:** None. **A. Tang:** None. **M. Zahran:** None. **S. Lam:** None. **J. Bonaventura:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Application 62/627,527 with the U.S. Patent Office.. **F. de Carvalho:** None. **C. Lacefield:** None. **M. Michaelides:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock in Metis Laboratories; Patent Application 62/627,527 with the U.S. Patent Office.. **S. Chan:** None. **O. Yizhar:** None. **C. Kellendonk:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.06

Topic: E.03. Basal Ganglia

Support: NIH R01MH110556
"la Caixa" Foundation Postgraduate fellowship

Title: Functional characterization of genetically defined subtypes of nigrostriatal dopamine neurons

Authors: *M. AZCORRA[1], R. AWATRAMANI[2], D. A. DOMBECK[3];
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Abstract: Recent single cell studies have identified several molecularly distinct subtypes of midbrain dopamine neurons (Poulin et al. 2016), with different cell body locations, projection patterns, and vulnerability in Parkinson's Disease models. But whether this genetic division relates to the function of the neurons remains unanswered. Here, we characterized the activity of several of these subtypes in the mouse during dopamine related behaviors, such as locomotion, reward consumption, and response to aversive stimuli. We used intersectional genetic strategies to label the individual dopaminergic subtypes with the calcium indicator GCaMP6f, and recorded changes in fluorescence using one-photon fiber photometry. We found that, beyond the previously identified morphological differences, nigrostriatal dopamine subtypes have distinct functional signaling patterns. For example, locomotion related calcium transients correlated mostly with accelerations in some subtypes, but mostly with decelerations in others. These results could explain previous incongruences in dopamine signaling patterns found during recordings of undefined dopamine neuron subtypes during movements (Howe et al. 2016, Dodson et al. 2016, Da Silva et al. 2016 Coddington et al. 2018), and have implications for the research and treatment of dopamine related disorders.

Disclosures: M. Azcorra: None. **R. Awatramani:** None. **D.A. Dombeck:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.07

Topic: E.03. Basal Ganglia

Title: Striosome circuitry and constraint on locomotor vigor with respect to an unlearned valence differential

Authors: *S. HAWES[1], **B. OLDHAM**[1], B. LIANG[2], D.-T. LIN[3], H. CAI[4];
[1]Natl. Inst. on Aging, Bethesda, MD; [2]Natl. Inst. On Drug Abuse, Baltimore, MD; [3]Behavior Neurosci., NIH NIDA IRP, Baltimore, MD; [4]Neurogenetics, Natl. Inst. Aging, Bethesda, MD

Abstract: Survival demands that safe and unsafe contexts are met with different locomotor profiles, yet whether striatal microcircuitry links contextual valence to spontaneous locomotor vigor is unresolved. Using a SepW1-Cre “Patch” model, we test for striosome contributions to spontaneous locomotion using ablation, calcium-imaging and chemogenetics in the context of a modified Light/Dark box test. We find that locomotor speed reflects the unlearned, external valence differential present in this test, and that striosomes gate valence-oriented speed selection. We also investigate circuit anatomy through regional synaptic density quantification in striatal output nuclei transfecting a cre dependent synaptophysin infused GFP. We observe denser direct pathway innervation in the internal pallidum, with more diffuse indirect pathway synapse formation. We provide a more intricate understanding of striosomes regulation of anxiogenic locomotor response, as well as patch circuit architecture that leads to complex striosome-related behavior.

Disclosures: S. Hawes: None. **B. Oldham:** None. **B. Liang:** None. **D. Lin:** None. **H. Cai:** None.

Poster

P522. Systems Behavior II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P522.08

Topic: E.03. Basal Ganglia

Support: Rutgers University Fund

Title: The role of the striatum in the formation of cortical attractor dynamics

Authors: *A. STETSENKO, T. Z. KOOS;
Rutgers, State Univ. New Jersey, Newark, NJ

Abstract: A recent study on working memory in the anterior lateral motor cortex (ALM) suggests that working memory is supported by discrete attractor dynamics [Inagaki et al., Nature 566(7743) 212-217, 2019]. However, it is not well understood which brain regions are involved in these dynamics. It is known that specific areas of the striatum are activated during the performance of tasks involving working memory [Akhlaghpour et al., Elife, 5, e19507, 2016]. In the current study, we explore the possibility that the striatum, and more broadly the basal ganglia, are integral parts of this attractor network. We use a working memory task that requires mice to give an appropriately chosen licking response (left or right reward port licking) 2 seconds after the termination of the discriminative auditory cue. We perform calcium imaging of task-related activity of pyramidal neurons in the ALM and examine the effects of bilateral and/or unilateral optogenetic manipulation of striatal activity. The optogenetic stimuli are timed to the beginning of the delay period. We observe effects of the manipulation on both the behavioral performance of the animals (more discrimination errors, i. e. left instead of right lick and vice versa) and on neuronal activity in the ALM (changes in the activity of the task selective and nonselective neurons). These results suggest the normal functioning of the basal ganglia is necessary for the performance of this task. And the basal ganglia may specifically contribute to the emergence or maintenance of the task-selective cortical attractor states. More specifically, we hypothesize that the striatum supports the maintenance of activity corresponding to the correct choice and suppresses the activity corresponding to the incorrect choice.

Disclosures: A. Stetsenko: None. **T.Z. Koos:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.01

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS116464

Title: Smart Dura: A monolithic functional surface array for chronic neural interfacing with non-human primates

Authors: *S. MONTALVO VARGO[1], T. BELLOIR[2], I. KIMUKIN[1], D. J. GRIGGS[2], J. REDDY[1], Z. AHMED[1], S. FISHER[2], A. YAZDAN-SHAHMORAD[2], M. CHAMANZAR[1]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Washington, Seattle, WA

Abstract: A neural interface that can be safely implanted for long-term recording and stimulation is highly desired for chronic monitoring of the brain's function and designing effective therapeutics. Despite the recent developments in surface micro-electrocorticographic (μECoG) arrays for rodents, the design of a stable, chronic interface for non-human primate (NHP) brain still remains elusive. In previous work, to keep the craniotomy open for successive acute experiments using surface ECoG arrays, artificial dura was introduced as a passive cap made of polydimethylsiloxane (PDMS) to cover the surface of the exposed brain in between the experiments. Since PDMS can be made optically transparent, artificial dura also provides optical access to the brain tissue.

Here, we are capitalizing on our previous work and demonstrate a novel neural interface design combining a chronic, transparent and flexible architecture inspired by the artificial dura that monolithically incorporates a high density of electrophysiological recording electrodes. Using thin-film microfabrication on flexible polymer substrates maintains the cranial pressure and minimizes tissue damage and at the same time reduces the chance of tissue infection. Our novel neural interface, called smart dura, can replace the native dura and provide neural recording and stimulation capabilities with high spatiotemporal resolution. Our current design features a honeycomb lattice for symmetrical recording and a channel count of 32 and 64 electrodes over a large area of NHP cortex (300mm^2). The resolution of our lithographic technique enables increasing the number of channels to 512 in each single thin-film layer. The transparent smart dura allows for a hybrid modality with external optical stimulation or imaging to augment the electrical recording and stimulation capability of smart dura. With 20-40 μm diameter electrodes, electrochemical impedance spectroscopy (EIS) measurements confirmed an average impedance of <1MΩ at 1 kHz for electrodes, thus enabling electrophysiological recording of multi-units and local field potentials with acceptable signal-to-noise ratio. We will discuss the details of our design, the microfabrication method and the in-vivo validation of the smart dura in the somatosensory cortex of macaques.

Disclosures: S. Montalvo Vargo: None. **T. Belloir:** None. **I. Kimukin:** None. **D.J. Griggs:** None. **J. Reddy:** None. **Z. Ahmed:** None. **S. Fisher:** None. **A. Yazdan-Shahmorad:** None. **M. Chamanzar:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.02

Topic: E.05. Brain-Machine Interface

Support: R01NS089688
U01NS113279
R01NS062019

Title: Chronic electrophysiological performance of L1CAM coated Utah arrays in non-human primate model

Authors: *D. SHI[1,3], A. B. SCHWARTZ[3,2], X. T. CUI[1,3];
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Abstract: By stimulating or recording from the microelectrodes chronically implanted in the brain, Brain-Computer Interface (BCI) technology can be used to restore lost sensations and motor functionalities. However, the performance of the intracortical electrodes will degrade significantly over time partially due to the inflammatory tissue response to those neural implants. As a biomimetic coating on the electrode surface, the neuroadhesive molecule L1CAM has demonstrated great potential in attenuating the foreign body response (FBR), promoting neuronal health and proximity. Application of the coating has prolonged chronic electrophysiological performance up to 16 weeks in rodent models (Asiyeh Golabchi et al., 2020). Here, we evaluated the chronic recording performance of the L1-coated 10-by-10 Utah arrays implanted in the motor cortex of Rhesus monkeys (n=3). After the non-human primate (NHP) subjects recover from the Utah array implantation surgery, two minutes of baseline neural activity was recorded every week. Manual unit clustering task was performed in the 3D PCA space using the Offline Sorter (Plexon). Some of the electrophysiological data from both L1-coated and un-coated arrays were assigned to a blinded operator to establish the manual unit sorting baseline. Next, "MountainSort" was chosen as a fully automated method for sorting the entire electrophysiology data set (Jason Chung et al., 2017). MountainSort input parameters (detection threshold, detection sign, etc.) were carefully tuned to be comparable to the manual sorting baseline and kept consistent throughout the sorting process. L1-coated arrays showed significantly higher unit amplitude (169.8±127.1 μV, p=0.0032, Welch's t-test) than the un-coated control arrays (84.52±49.05 μV) for the first five months and retained a higher trend than the un-coated arrays for another 15 months. This result reinforced existing evidence supporting the beneficial effect of L1CAM in improving chronic recording performance. Aside from neuronal recording data, monthly electrochemical impedance spectroscopy (EIS) data showed that coated arrays have overall lower impedance than the uncoated control arrays. Ongoing immunohistochemical analysis of the brain tissue and Utah array explants will provide a more comprehensive understanding of the biological and material factors affecting the chronic interface and uncover the mechanism of action of the L1CAM coating in primates. The ability of L1CAM coating to improve neural recording yield and longevity will benefit both BCI technologies and neuroscience studies relying on chronic recordings.

Disclosures: D. Shi: None. **A.B. Schwartz:** None. **X.T. Cui:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.03

Topic: E.05. Brain-Machine Interface

Support: T32 Fellowship in Recovery and Restoration of CNS Health and Function

Title: Tracking the migration of free-floating microdevices injected into the brains of rodents using Magnetic Resonance Imaging

Authors: *A. KHALIFA[1], J. WEIGAND-WHITTER[1], C. FARRAR[1], A. EISAPE[2], S. CASH[1]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Free-floating implantable neural interfaces are an emerging powerful paradigm for mapping and modulation of brain activity. Wirelessly powered microdevices are being miniaturized to improve safety, longevity, and spatial resolution in a wide range of biomedical applications. Some of them have reached a point where they can be injected whole into the central nervous system. However, these intracortical microdevices are faced with two vital questions. 1) How can they be injected into the brain rapidly in an essentially arbitrary location? 2) Will the implants migrate over time? To answer the first question, we have built a simple injection tool that allows for precise, fast, and minimally invasive insertion into the central nervous system. To validate the needle injectable tool and the presented delivery approach, we evaluate the spatial precision and rotational alignment of the microdevices injected into agarose and brain with the aid of tissue clearing techniques and MRI. To answer the second question, we tracked microdevices injected in different areas of the brain of five rats over 5 months using a 9.4T MRI scanner. We then present immunohistology data to assess the damage caused by the injection tool and the chronic immune reaction to the microdevices. By virtue of its simplicity, we show that the proposed injection method can be used to inject microdevices of all sizes and shapes (340 × 330 × 250 µm; 300 × 200 × 80 µm, 200 × 200 × 250 µm) and will do so in a fast (< 70 sec per microdevice) and minimally-invasive manner. Furthermore, MR images show that microdevices smaller than 0.01 mm^3 remain anchored in the brain at the targeted injection over 5 months. Based on CD68 (microglia) and GFAP (astrocytes) immunoreactivity to the microdevice, we hypothesize that glial scar formation is preventing the migration of chronically injected microdevices in the brain. The findings of this research show a promising future for these next-generation types of intracortical electrodes in animal models.

Disclosures: A. Khalifa: None. **J. Weigand-Whitter:** None. **C. Farrar:** None. **A. Eisape:** None. **S. Cash:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.04

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01DC008358

Title: Unsupervised Channel Compression Methods in Neural Prostheses Design

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Abstract: High-performance brain machine interfaces (BMIs) require scaling recording channel count to enable simultaneous recording from large populations of neurons. Unfortunately, proposed implantable neural interfaces have power requirements that scale linearly with channel count, introducing a critical constraint on the number of channels that can be recorded simultaneously. To facilitate the design of interfaces with reduced power requirements, we develop an unsupervised-learning-based compressed sensing strategy. This strategy suggests novel neural interface architectures which compress neural data by methodically combining channels of single-unit spiking activity. We develop a compression strategy that models the neural population activity as being generated from a lower dimensional set of latent variables and aims to minimize the loss of information in the latent variables due to compression. This strategy assumes that the low-dimensional latent variables effectively describe the activity of a high-dimensional population of neurons. Previous work demonstrated that low-dimensional latent variables can describe neural variability effectively and enable better performance in online BMIs than features based on high-dimensional neural observations (Kao et al. 2015). Neural population dynamics, as described by low-D latent variables, can be accurately estimated from unsorted multi-units (Trautmann et al. 2019). That study connects unsorted multi-units activity to the theory of random projections and compressed sensing. Multi-units can be emulated as a random projection of single units and as demonstrated these random projections can be used to infer low-D latent variables without the need to measure the activity of every single unit. This provides motivation to study whether informed mixtures of single-unit features, based on designed compression methods, can yield high accuracy estimates of low-D latent variables with even lower numbers of channels. We introduce two evaluation metrics to assess the latent variables inferred from compressed features to quantify the loss of information. We measure the accuracy with which the activity of held-out neurons can be estimated and the accuracy with which the behavior can be estimated. We apply these methods to data collected from the RA region in a zebra finch in a free vocal behavioral experiment (Tostado, SfN 2021) and compare the compression strategy to a random projections strategy. Our initial results suggest that the

compression strategy can reach the similar prediction error of the activity of held-out neurons to random projections with a 50% reduction in dimensionality.

Disclosures: A. Alothman: None. **P. Tostado:** None. **Z. Arneodo:** None. **T. Gentner:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); V.G. holds shares in Neuralink, Corp., and Paradromics, Inc. These organizations had no role in study design, data collection and analysis, decision to publish, or preparation of abstract. F. Consulting Fees (e.g., advisory boards); V.G. currently consults for Paradromics, Inc. They had no role in the study design, data collection and analysis, decision to publish, or preparation of the abstract.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.05

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS092950001
NIH Grant T32 NS047987

Title: The effect of subthalamic nucleus deep brain stimulation and volume of tissue activated on sequential memory-guided reaching encoded with peripheral vision versus proprioception

Authors: F. J. DAVID[1], *Y. RIVERA[2], T. ENTEZAR[4], M. MUNOZ[1], Q. DRANE[1], G. PAL[5], L. V. METMAN[6], S. SANI[6], J. ROSENOW[7], A. S. KURANI[8], D. M. CORCOS[3]; [2]Physical Therapy and Human Movement Sci., [3]Dept. of Physical Therapy/Human Movement, [1]Northwestern Univ., Chicago, IL; [4]Univ. of Illinois Urbana Champaign, Champaign, IL; [5]Rush Univ. Med. Ctr., Chicago, IL; [6]Rush Med. Ctr., Chicago, IL; [7]Northwestern Med., Chicago, IL; [8]Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Peripheral-visual and proprioceptive inputs are used to encode spatial locations as well as plan and execute movements to sequentially memorized locations. These inputs recruit the basal ganglia-thalamo-cortical network which degenerates in Parkinson's disease (PD). Previously, we studied the effect of anti-Parkinsonian medication on sequentially memory-guided reaching amplitude, velocity, and error when encoded with either peripheral vision or proprioception. Medication had no effect on reaching amplitude, improved velocity but did not normalize to healthy controls (HC), and reduced error further from HC levels. Here, we studied the effect of subthalamic nucleus deep brain stimulation (STN DBS) on sequential memory-guided reaching. We asked 2 questions: 1) How does STN DBS affect sequential memory-guided reaching amplitude, velocity, and error when encoded with peripheral-vision vs

proprioception, and 2) does the VTA intersection with the STN (VTA-STN overlap) predict sequential memory-guided reaching amplitude, velocity, and error in each of the 2 experimental paradigms? Participants with PD who underwent bilateral STN DBS (n=15, m=15) and HC (n=13, f=2) performed 2 memory-guided reaching tasks: 1) encoding targets through peripheral vision and 2) through proprioception. During the peripheral-visual paradigm, participants encoded 3 sequential LED targets to memory, and then reached to the memorized locations as accurately as possible, using peripheral vision. The proprioceptive paradigm was similar, except participants were blindfolded throughout the task and an experimenter guided their relaxed arm to 3 target locations for encoding. Trials were conducted in total darkness over 2 days for participants with PD and 1 day for HC. Participants with PD performed the tasks off medication while ON and OFF bilateral STN DBS. We estimated VTA-STN overlap using Lead DBS. Dependent outcomes were reaching amplitude, velocity, and error. In contrast to our medication findings, STN DBS significantly increased reaching amplitude and velocity towards HC in both paradigms, but did not normalize these values. STN DBS had no effect on peripheral-visual reaching error and caused proprioceptive error to move closer to HC. VTA-STN overlap did not predict any reaching outcome. These findings suggest that the mechanism of action of STN-DBS differs from medication and VTA-STN overlap did not drive this improvement. Future research should determine the mechanism by which VTA of non-STN areas cause this improvement.

Disclosures: F.J. David: None. **Y. Rivera:** None. **T. Entezar:** None. **M. Munoz:** None. **Q. Drane:** None. **G. Pal:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute of Neurological Disorders and Stroke, Bakalar Family Foundation, and Parkinson's Foundation. F. Consulting Fees (e.g., advisory boards); Asim, Huron, AbbVie, Boston Scientific, KeyQuest, Qessential, SeaGrove, Medtronic, and Abbott. **L.V. Metman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute of Health, Abbott, AbbVie, Boston Scientific, Biogen, Medtronic, Neuroderm, and Prilenia Therapeutics. **S. Sani:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute of Health, Medtronic, Abbott, Boston Scientific. **J. Rosenow:** F. Consulting Fees (e.g., advisory boards); Boston Scientific. **A.S. Kurani:** None. **D.M. Corcos:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute of Health and Michael J. Fox Foundation. F. Consulting Fees (e.g., advisory boards); National Institute of Health.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.06

Topic: E.05. Brain-Machine Interface

Support: University of Toronto CARTE Seed Grant

Title: Reducing convolutional neural network architectures for selective peripheral nerve recording on implantable devices

Authors: *Y.-C. HWANG[1,4], R. GENOV[2], J. ZARIFFA[1,4,3,2];
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Abstract: Background: Closed-loop control of functional electrical stimulation involves using recorded nerve signals to make decisions regarding nerve stimulation in real-time. Surgically implanted devices that can implement this strategy have significant potential to restore natural movement after paralysis and improve the quality of life of individuals living with spinal cord injury. Discriminating between recorded signals from different neural pathways can provide information about the body's state and external inputs, which can then be used to decide on the most appropriate form of stimulation to deliver. Our previous work demonstrated the use of a convolutional neural network (CNN), ESCAPE-NET, to discriminate spatiotemporal signatures produced by different neural pathways. Despite state-of-the-art performance, this approach required too much data storage, power and computation time for implementation on hardware that is small enough to be surgically implanted in patients and fast enough for real-time decision making. **Objective:** This study aimed to minimize resource consumption while maintaining performance accuracy for machine learning techniques that can distinguish between neural pathways in high-density multi-contact nerve cuff electrode recordings. **Methods:** Several CNN architectures were evaluated using a dataset of rat sciatic nerve recordings previously collected using 56-channel (7 x 8) spiral nerve cuff electrodes. The CNNs were trained to classify individual, natural compound action potentials (nCAPs) elicited by ankle dorsiflexion, plantarflexion or heel prick. Reduced-complexity CNN architectures have been explored and designed using techniques such as dropout and fully convolutional networks (FCN). **Results:** Reduced ESCAPE-NET using dropout was 16x smaller than the original architecture and operated with 2.4x fewer floating-point operations per second (FLOPS), performing with 80.6% accuracy compared to a baseline of 80.7%. Reduced ESCAPE-NET using FCNs was 420x smaller than the original and operated with 3.5x fewer FLOPS, performing with 77.8% accuracy compared to a baseline of 80.7%. **Conclusion:** These reduced versions of ESCAPE-NET require significantly fewer resources with minimal accuracy loss, and thus will be more easily incorporated into a surgically implantable device that performs closed-loop control of stimulation in real-time. Beyond the impact on people with paralyzed limbs and spinal cord injury, this selective peripheral nerve recording technology has applications in the control of

prosthetic limbs and neuromodulation interventions for diseases such as chronic pain, diabetes, or incontinence.

Disclosures: Y. Hwang: None. **R. Genov:** None. **J. Zariffa:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.07

Topic: E.05. Brain-Machine Interface

Support: NSF CPS Grant 1739800
VA Merit Review A2275-R
NIH Grant 5T32EB004314-15
NIH Grant R01 NS040547-13

Title: Iterative Learning Control and Balance Control for Walking in a "Muscles-First" Motor-Assisted Hybrid Neuroprosthesis

Authors: S. HNAT[1], R.-D. REYES[1], M. NANDOR[2], N. MAKOWSKI[3], M. AUDU[1], R. TRIOLO[1], *R. QUINN[2];
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Abstract: Paralysis due to spinal cord injury (SCI) can limit mobility and result in secondary health impacts such as muscle atrophy and bone density loss. While commercial powered exoskeletons can restore mobility to individuals with SCI, we have developed a "Muscles-First" Motor-Assisted Hybrid Neuroprosthesis (MAHNP) to better counteract disuse atrophy. MAHNP stimulates the user's muscles to complete movements via functional neural stimulation (FNS) while electric motors assist as needed. Conventional orthotics are used for the trunk and shank, while each joint features its own separate power module with a brushless DC motor, electromagnetic solenoid brake, and harmonic drive transmission. MAHNP can be adapted for the user's anthropometry, with the power units connected by aluminum tubing with customizable limb length and pyramid adaptors that allow 15° of fitment adjustment in the sagittal and coronal planes. FNS is administered through implanted and/or surface electrodes capable of exciting hip, knee, and ankle flexion/plantarflexion and trunk flexion. We developed a biologically-inspired iterative learning controller (ILC) that swing the legs in a ballistic fashion. The control algorithm was tested using an anatomically realistic 3D musculoskeletal model of the lower leg and pelvis suitably modified to account for exoskeletal inertia. The model was developed and tested with the OpenSim biomechanical modeling suite. Preliminary data demonstrate controller efficacy via

swing-leg simulations and its ability to learn to balance muscular and motor contributions to improve performance and accomplish consistent stepping. In particular, the controller took 15 iterations to achieve the desired outcome with 0.3% error. Additionally, to automatically maintain balance while wearing MAHNP, we developed a method for estimating COM kinematics (typically used as feedback for balance controllers) using only body segment accelerations using a COM Estimation Algorithm. The algorithm was trained by minimizing the mean-squared-error between COM displacements measured by a gold-standard motion capture system and acceleration signals obtained from ten body-mounted IMUs on five able-bodied participants during unexpected and volitional destabilizing perturbations. We have tested the ILC on a participant with SCI wearing MAHNP and have begun balance control implementation in able-bodied participants wearing actuated ankles. Preliminary results suggest MAHNP 1) can improve with each step by maximizing muscle recruitment and 2) has the appropriate hardware (ankle plantarflexion torque and angular velocity) for maintaining balance.

Disclosures: S. Hnat: None. **R. Reyes:** None. **M. Nandor:** None. **N. Makowski:** None. **M. Audu:** None. **R. Triolo:** None. **R. Quinn:** None.

Poster

P549. Neurophysiology: Implanted Electrodes and Other Direct Interactions with Neurons V

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P549.08

Topic: E.05. Brain-Machine Interface

Support: JHUAPL Internal Research and Development

Title: Exploring vibrotactile and intracortical microstimulation feedback on sensory embodiment and expectations

Authors: L. E. OSBORN[1], ***A. C. G. CREGO**[1], B. P. CHRISTIE[1], D. A. D'ALMEIDA[1], D. P. MCMULLEN[3], C. FIRESTONE[4], F. V. TENORE[5], M. S. FIFER[2];
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Abstract: Artificial sensory feedback can provide useful information to users of external devices, such as prosthetic limbs. For prosthesis users, restored sensory information is useful for improving grasping function and enhancing perceived levels of device embodiment (i.e., defined here as integrating the device into one's internal body representation). Qualitative surveys are used to capture broad perceptual experiences pertaining to changes in embodiment. This integration relies on multiple factors, including perceived agency, ownership, and the sensory

stimuli of the system. In this study, we explored the role of artificial tactile feedback in promoting embodiment of a virtual robotic hand shown on a monitor. We provided either vibrotactile stimulation or intracortical microstimulation (ICMS) to the hand of a human participant who was implanted with microelectrode arrays in the primary somatosensory cortex (S1) and the motor cortex (M1). In the experiment, a virtual sphere made contact with a virtual robotic hand while the participant observed and received artificial stimulation in the same location as the touch occurring on the virtual hand. After an initial training period of 60 trials with simultaneous virtual touch and stimulation, we randomly introduced stimulation dropout trials at an effective rate of 1:15. The experiment was repeated for both vibrotactile stimulation on the thumb and ICMS to S1 regions mapped to the thumb. In initial exploratory sessions, the participant reported they were able to feel as if the tactile sensation was originating as a result of the virtual touch event, despite the virtual hand being spatially incongruent and physically located beyond the body. Using button presses to indicate a perceived tactile sensation, the participant had a false positive rate (i.e., pressed the button during a dropout trial) on 70% of the ICMS dropout trials and 53% of the vibrotactile stimulation dropout trials; however, after approximately half of those false positive trials the participant realized the absence of sensory information and reported having incorrectly pressed the button. Despite the absence of sensory stimulation, the behavioral response indicating perceived tactile sensation suggests the development of sensory expectations and embodiment of the external system. This work has implications for better understanding artificial sensory feedback in modulating levels of system embodiment. By quantifying the relationship between sensory events of an external system and user perception, we can develop intuitive stimulation paradigms that more readily promote human-machine teaming.

Disclosures: L.E. Osborn: None. **A.C.G. Crego:** None. **B.P. Christie:** None. **D.A. D'almeida:** None. **D.P. McMullen:** None. **C. Firestone:** None. **F.V. Tenore:** None. **M.S. Fifer:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.01

Topic: F.01. Neuroethology

Support: NIH F32NS112557
NIH R01NS102488
NIH R01NS108424
NIH R01DC014364
NIH UF1NS115821
NIH R21DC016340
HCA-A-1704-01747 Chan Zuckerberg Initiative

Title: Spatial and cellular transcriptomic mapping of the songbird brain provides context for the origin of vocal learning circuits

Authors: *D. P. MERULLO, G. KONOPKA, T. F. ROBERTS;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Songbird brains contain specialized regions with well-characterized connectivity for learning and producing complex vocalizations. Most of these song regions reside in the dorsal ventricular ridge (DVR), the largest portion of the avian forebrain. Molecular, anatomical, and electrophysiological specializations distinguish these song regions from surrounding portions of the DVR, suggesting that song learning may have arisen in parallel with the development of unique cell-types and/or through the re-organization and specialization of pre-existing cell-types within the DVR. To unambiguously characterize the cell-types of the DVR, we performed single-nuclei sequencing inside and outside of song regions of adult male zebra finches. We integrated all data and performed an unbiased clustering analysis to identify globally distinguishable cell-types. We report that glial cells and inhibitory GABAergic neurons are common to all DVR regions examined, showing no distinctions across neuroanatomical subdivisions or within song-specialized regions. However, the excitatory glutamatergic neurons are specific to sub-fields of the DVR, including the nidopallium and arcopallium. Nonetheless, the glutamatergic neuron classes in song regions do not differ from those in adjacent portions of the DVR, despite numerous gene expression differences. This indicates that each excitatory projection cell-type in a song region has a counterpart in the surrounding portions of the DVR. To further examine organizational principles and specializations of the DVR and other regions of the songbird brain, we performed spatial transcriptomics on whole sagittal tissue slices in adult male zebra finches. This technique maps the entire transcriptome while preserving spatial coordinates (but not at single-cell resolution). Using spatial coordinates as anchors, we computationally mapped differential gene expression patterns throughout the brain. Preliminary analysis reveals that spatial transcriptomic profiles clearly define anatomical sub-regions of the avian pallium and subpallium. While song regions largely conform to the transcriptional profiles of their adjacent regions, molecular specializations can be used to distinguish their borders from surrounding DVR fields. By integrating spatial and cellular transcriptional data, we aim to provide a general resource and framework for understanding the cell-type composition of the avian brain and the sub-regions responsible for orchestrating complex suites of behavior, like song learning. Our current results indicate that neurons within song regions largely reflect the cell-types found within the broader DVR.

Disclosures: D.P. Merullo: None. **G. Konopka:** None. **T.F. Roberts:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.02

Topic: F.01. Neuroethology

Support: University of Arizona BIO5 Institute Accelerate for Success
University of Arizona Core Facilities Pilot Program
National Science Foundation Graduate Research Fellowship
University of Arizona Marshall Foundation Dissertation Fellowship
University of Arizona Neuroscience and Speech, Language and Hearing Startup Funds
Graduate Interdisciplinary Program in Neuroscience

Title: An analysis of vocalization-associated molecular pathways and gene networks within the basal ganglia of mouse and finch Parkinson's disease models

Authors: C. A. MEDINA[1,2], S. J. MUNGER[1], M. CORENBLUM[3], J. L. ECKHARDT[1], L. MADHAVAN[2,3,4,5], J. E. MILLER[1,2,3,5,6];
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Abstract: Parkinson’s Disease (PD) is the most prevalent neurodegenerative disorder worldwide that affects motor control. Limb motor symptoms include loss of balance, resting tremor, and rigidity that present during the later stages after significant neurodegeneration has occurred. Unlike limb motor symptoms, vocal motor symptoms such as a soft, monotonous voice and changes in speaking rate can occur earlier in the disease progression. Human studies suggest that deterioration of basal ganglia function linked with limb deficits are also linked with this worsening vocal quality. However, very little is known about the molecular mechanisms of basal ganglia dysfunction that underlie the early vocal changes in contrast to later stage limb deficits. Prior research showed that driving overexpression of the causally-related PD gene wild-type human *SNCA* - which encodes for the neuronal protein alpha-synuclein (αsyn) - with the Thy-1 promoter in mice leads to quieter and shorter ultrasonic calls along with deterioration in vocal structure. Interpreting these findings, however, is difficult given the lack of understanding regarding the neurobiological mechanisms involved in mouse vocalizations. In contrast, the neurobiology of vocal control in zebra finch is well-characterized and strongly resembles human speech pathways from the molecular through systems level. Recently, we showed that viral-mediated overexpression of human αsyn in a vocal-dedicated central brain circuit that includes the basal ganglia causes deterioration of finch song - it becomes quieter, shorter, and they sing less. Here, we leverage both the Thy1-αsyn mouse and our viral-mediated finch PD model to identify differentially expressed genes, molecular pathways, and genetic networks that are perturbed within the basal ganglia under vocalizing and quiet behavioral states. We first compared mouse ultrasonic vocalizations to finch song using the machine learning program DeepSqueak. Secondly, we conducted bulk mRNA-seq of the basal ganglia from vocalizing and quiet finch and mice to identify genetically conserved vocal pathways using differential gene expression (DGE) and Weighted Gene Coexpression Network (WGNCA) analyses on control

mice and finches. DGE was used to identify individual gene targets or molecular pathways, and WGCNA was used to construct vocalization-associated gene networks. Lastly, vocalization-associated genetic networks from control animals were compared to networks derived from both Parkinsonian models. Our experiments represent the first comparative transcriptomic study conducted to isolate vocalization-associated genetic networks within a disease framework.

Disclosures: C.A. Medina: None. **S.J. Munger:** None. **M. Corenblum:** None. **J.L. Eckhardt:** None. **L. Madhavan:** None. **J.E. Miller:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.03

Topic: F.01. Neuroethology

Support: NIH Grant

Title: Micro RNA miR-9 regulates Area X neuron structures in zebra finches

Authors: *H. JARRELL[1], Z. HUANG[2], Y. DING[3], Z. SHI[2], X. LI[4];
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Abstract: Male juvenile zebra finches learn to sing in a manner parallel to young humans learning to speak. Area X, the avian basal ganglia, plays important roles in song related sensory-motor learning and performance in zebra finches. We have recently shown that expression of miRNA miR-9 is regulated in Area X during song development and that overexpression of miR-9 in Area X of juveniles leads to impairments in vocal learning and performance. We have also shown that miR-9 regulates FOXP2 (a gene associated with speech and language development) and many FOXP2 downstream genes that are important for neuronal structure development and plasticity. These observations prompted us to hypothesize that miR-9 modulates neuronal structure in Area X. In this study, we examined miR-9's role in modulating dendritic and spine structure of the spiny neurons in Area X of juvenile and adult zebra finches. We made a lentiviral vector that expresses miR-9 and a fluorescent marker, mCherry. We stereotaxically injected the miR-9 expressing lentivirus into the Area X of one hemisphere and a control virus into Area X of the contralateral side of 27 (±3) day old juveniles. We sacrificed the animals at 60 days (juvenile) and 100 days of age (adult), imaged virally labeled spiny neurons, which were identified by DARPP-32 staining. We traced three-dimensional neuronal dendritic structures, and quantified dendritic length, dendritic complexity and spine structures of spiny neurons. We found that miR-9 overexpression in juvenile Area X reduced dendrite length and dendrite complexity and reduced spine density of spiny neurons. In addition, miR-9 downregulated DARPP-32, a key

molecule in dopamine signaling. These changes were long lasting and persisted into adulthood. These results provide strong evidence for miR-9's role in regulating basal ganglia neuronal structure. Since the basal ganglia is key to many neurological and psychiatric disorders in humans, dysregulation of miR-9 may contribute to these disorders as well.
Key words – basal ganglia, Area X, medium spiny neurons, dendrite, spines, zebra finches

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

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.04

Topic: F.01. Neuroethology

Title: Area X Marks the Spot: Cell-Type-Specific Gene Expression Signatures of Sensorimotor Learning in the Zebra Finch

Authors: *E. COOKE[1], S. A. WHITE[2];
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Abstract: The courtship song of the zebra finch (*T. guttata*) is a complex, quantifiable, and highly motivated behavior that provides the opportunity to study neural mechanisms underlying sensorimotor learning and performance. Young male birds learn their songs during partially overlapping sensory and sensorimotor learning stages in development, during which songs from an adult tutor are internalized and then imitated with increasing fidelity. The brain regions required for song learning, including song-dedicated basal ganglia Area X, have highly convergent gene expression patterns to human forebrain regions involved in speech learning. In Area X, the language-linked transcription factor FoxP2 is expressed in a tightly regulated manner necessary for optimal song learning: expression levels are high at the start of the morning and subsequently decrease over the next 2 hours proportionally to the amount a bird sings, while song variability increases. Our previous work used weighted gene co-expression network analysis (WGCNA) to identify transcriptional expression patterns involved in song learning and production; notably, genes in a module linked with singing were found to overlap heavily with human datasets from individuals with autism and language impairment in a follow-up meta-analysis (Lombardo et al, 2018). However, as the WGCNA was done using bulk sequencing data, it is unknown which cell type(s) are regulated following singing. Droplet-based single cell RNA sequencing with the 10XGenomics platform allows for investigation into cell-type-specific changes in gene expression between singing and non-singing siblings. This data aids our understanding of how distinct cell types within the basal ganglia circuit contribute to motor learning, and specifically which basal ganglia cell types are differentially regulated after singing,

in and outside of the sensorimotor learning phase. We additionally apply a deconvolution analysis to our previous bulk dataset based on newly reported Area X cell types from frozen nuclei tissue, to reinforce our findings.

Disclosures: E. Cooke: None. **S.A. White:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.05

Topic: F.01. Neuroethology

Support: NIH GM120464
NSF IOS1456302

Title: Molecularly-defined cell types of the zebra finch deep cortical layer analogs

Authors: *A. NEVUE[1], C. V. MELLO[2];
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Abstract: Songbirds learn their songs via tutor imitation and possess a specialized brain circuitry to support this behavior. Despite considerable knowledge on the anatomical, physiological, and molecular properties of the songbird vocal circuitry, especially in zebra finches, our understanding of how song nuclei relate to their surrounding brain regions in terms of their cellular type composition is limited. The robust nucleus of the arcopallium (RA) is a particularly prominent and extensively studied vocal nucleus in zebra finches. It is the major forebrain vocal output nucleus and it plays important roles in encoding acoustic features of song. RA is located in the intermediate arcopallium (AI), a brain region thought to contain avian analogs of projection neurons of deep layers of motor and sensory cortices in mammals. We have previously reported that RA shares a large portion of gene expression specializations with the adjacent dorsal intermediate arcopallium (AId), a portion of the AI that has been linked to somatic motor function. However, the extent to which the shared molecular profile of RA and AId reflects a shared cell type composition is largely unknown. Defining the cell types in the intermediate arcopallium (AI) can give insights into the function and evolution of RA as a specialized nucleus for vocal-motor control. To determine the cell types present in AI, we microdissected a core portion of the AI that includes RA, AId, and the adjacent ventral AI (AIv), an auditory region, in adult male zebra finches. We isolated nuclei and performed single nuclei RNA sequencing on the 10x Genomics platform, and performed follow-up *in situ* hybridizations to verify the spatial distribution of genes of interest. We identified cell class clusters associated with neuronal (54% of total nuclei) and non-neuronal (46% of total nuclei) cells. While

excitatory cells (*SLC17A6+*) were non-differentially distributed throughout the AI, some subclasses were specific to the individual nuclei, most prominently an RA-specific class of excitatory cells labeled by the androgen-related gene *SRD5A2*. GABAergic cells (*GAD2+*), in contrast, were fewer in number in RA compared to AId and AIv, with suggestive evidence of subclasses based on classical inhibitory neuron-related genes such as *PENK*, *NPY*, and *LAMP5*, although most of these were largely distributed throughout the AI. Future work will further characterize the cell types of the AI to gain insights into the specialized vs shared properties of its component nuclei, and of the genes that define specific cell types within these nuclei.

Disclosures: A. Nevue: None. **C.V. Mello:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.06

Topic: F.01. Neuroethology

Support: R01 NS075044

Title: Establishing behavioral relevance to molecularly defined GABAergic interneurons within a songbird premotor circuit

Authors: *E. HOZHABRI[1,2], A. CORREDERA ASENSIO[1], M. ELMALEH[1,2], P. W. FRAZEL[1], J. DIMIDSCHSTEIN[3], G. J. FISHELL[4], M. A. LONG[1];
[1]Neurosci. Inst., NYU Sch. of Med., New York, NY; [2]New York Univ., New York, NY; [3]Broad Inst., Cambridge, MA; [4]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: GABAergic interneurons enrich the computational power of neural circuits, with different subpopulations often proposed to play distinct roles within a network. In the mammalian brain, separate categories can be defined based on their morphological, physiological, molecular, and connectivity profiles. Although these classes have been well-characterized in sensory cortices, the roles of distinct inhibitory cell types in the production of complex motor behaviors are still poorly understood. To address this issue, we investigated the role of GABAergic interneurons within the zebra finch song production pathway, which has the distinct advantage of a complex, but highly tractable, behavior mediated by a set of well-characterized brain regions. In a key premotor locus, called HVC, inhibition mediated by local circuit interneurons plays several distinct roles in song learning and production. Furthermore, we and others have characterized heterogeneity within the HVC interneuron population. However, the degree to which these diverse profiles mediate distinct roles within the HVC circuit remains unclear.
We begin to address this issue by characterizing molecularly defined interneurons across a range

of behaviors. We first explored the genetic diversity of HVC interneurons using single-nuclei transcriptomics and found distinct clusters of HVC interneurons consistent with recently published findings (Colquitt et al., 2021). We then categorized electrophysiological responses of HVC interneurons in different behavioral contexts using chronically implanted silicon probes. To link these observations, we used *in vivo* 2-photon targeted recordings of HVC interneurons labeled with GFP under the control of the mDlx enhancer (Dimidschstein et al., 2016) followed by post hoc immunolabeling with antibody markers for major transcriptomically defined interneuron subclasses. Using this approach, we have begun to relate specific physiological roles of interneurons with their molecular designations.

Disclosures: E. Hozhabri: None. **A. Corredera Asensio:** None. **M. Elmaleh:** None. **P.W. Frazel:** None. **J. Dimidschstein:** None. **G.J. Fishell:** None. **M.A. Long:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.07

Topic: F.01. Neuroethology

Support: NSF Bio Anthro DDRIG 1613709
Doctoral Year Fellowship - Graduate Division - UCLA
Trainee Professional Development Award (TPDA) - SfN
Eureka and Hyde Fellowship - Department of Integrative Biology and Physiology – UCLA
International Peace Scholarship – Philanthropic Educational Organization (PEO)
Will Rogers Scholarship - Center for Accessible Education – UCLA
Coordination for Improvement of Higher Education Personnel (CAPES), Brazil

Title: Neurogenetic Mechanisms for Vocal Learning, Practice, and Performance

Authors: *M. FARIAS-VIRGENS[1], T. DEACON[3], S. A. WHITE[2];
[2]Integrative Biol. & Physiol., [1]Univ. of California Los Angeles, Los Angeles, CA; [3]Univ. of California at Berkeley, Berkeley, CA

Abstract: We have previously used zebra finches (ZF; *Taeniopygia guttata*) to uncover hundreds of genes whose brain co-expression patterns are correlated with vocal learning (e.g., the pupil's song spectral and sequential similarity to its tutor's song) and performance (e.g., amount of singing and song stereotypy - rhythmic, repetitive, fixed, predictable). Gene co-expression in young birds' brains, which are actively engaged in song learning, can be correlated with both learning and singing-related variables. Noticeably, gene modules correlated with song learning are broadly distributed across the songbird basal ganglia, comprising both the song-

dedicated nucleus Area X of the basal ganglia and the adjacent, more generalist, region of the ventral striatopallidum (VSP), while singing-related gene modules are uniquely found in Area X. At later ages, when ZFs have passed the vocal learning phase and are instead engaged in song maintenance and performance, learning modules are disassembled and co-expression exclusive to singing persists, which may characterize a transcriptional dynamic underlying the closure of a critical period in which the bird can change its song. We now build upon this work by assessing the extent to which these spatiotemporal patterns of co-expression of behaviorally relevant gene modules found in the ZF are preserved in the Bengalese finch (BF: *Lonchura striatta domestica*), a songbird species with a remarkably more complex song, in which transitions between vocal units are weakly fixed, introducing variability in song sequencing. For this, we have collected song and transcriptomic data from Area X and VSP of young and adult male BFs. Additionally, to analyze a bird's ability to adaptively modulate its vocal variability and identify the associated transcriptional changes, we compared male BFs singing in natural contexts known to elicit differences in song stereotypy. In BFs, a species in which only males learn to sing, social context modulates song variability: it is typically reduced when a male sing to females (FD; female directed) relative to when he sings alone (UD; undirected). Accordingly, within our adult cohort, song and brain transcriptomic data were collected from male BFs after a period of continuous singing either alone or in the presence of a female. Results from our work illuminate context-dependent and spatiotemporal transcriptional networks in the avian basal ganglia that contribute to motor learning and reinforcement and have the potential to highlight specializations related to complex communication both in birds as well as in our own species.

Disclosures: M. Farias-Virgens: None. **T. Deacon:** None. **S.A. White:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.08

Topic: F.01. Neuroethology

Support: NSERC Discovery Individual to LSP

Title: Seasonal changes in neurogenesis and indicators of stress in European starlings (Sturnus vulgaris)

Authors: S. D. T. AITKEN[1], M. M. SOLLOWS[1], A. OPREA[1], C. A. BARBER[3], ***L. S. PHILLMORE**[2], B. M. B. PARKS[1];
[1]Psychology and Neurosci., [2]Dalhousie Univ., Halifax, NS, Canada; [3]Biol., St. Mary's Univ., Halifax, NS, Canada

Abstract: During breeding season, male songbirds, like the European starling, sing stereotyped, complex songs to attract mates, and females use these complex songs to judge male fitness. Following breeding season males vocalize less frequently and their songs become more variable. Accompanying these changes in vocal behaviour are changes in neurogenesis, the process by which neurons are born, differentiate, migrate, and are incorporated into neural circuits in the brain. We examined seasonal differences in neurogenesis in three brain regions associated with vocal learning, performance, and discrimination: HVC, caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM). We captured male and female starlings in spring and fall and counted neurons in these regions using an endogenous marker of neurogenesis, doublecortin. We also examined seasonal differences in stress using two blood measures: plasma corticosterone levels and heterophil/leukocyte ratio. Male birds had more HVC neurogenesis in fall than in spring, and female birds had more HVC neurogenesis than males. We also found a positive relationship between corticosterone levels and neurogenesis in HVC and NCM, suggesting that stress may modulate aspects of neurogenesis in specific auditory brain regions.

Disclosures: S.D.T. Aitken: None. **M.M. Sollows:** None. **A. Oprea:** None. **C.A. Barber:** None. **L.S. Phillmore:** None. **B.M.B. Parks:** None.

Poster

P583. Vocal/Social Communication: Avian I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P583.09

Topic: F.01. Neuroethology

Support: NSERC Discovery individual to LSP

Title: A novel classification method to quantify adult neurogenesis using doublecortin (DCX) in songbirds

Authors: *B. M. B. PARKS[1], C. A. STADNYK[1], R. M. W. GIBBS[1], S. D. T. AITKEN[1], A. V. KINGBÉDÉ[2], S. MOORE[2], M. F. GUIGUENO[2], L. S. PHILLMORE[1];
[1]Psychology and Neurosci., Dalhousie Univ., Halifax, NS, Canada; [2]Biol., McGill Univ., Montréal, QC, Canada

Abstract: The microtubule-associated protein doublecortin (DCX) has been long used as an immunohistochemical marker of neurogenesis during development in rodents and was proposed as a marker of neurogenesis in songbirds by Boseret et al. (2007), and later by Balthazart et al. (2008) and Balthazart and Ball (2014). Boseret et al. (2007) described four distinct cellular morphologies arising from DCX immunolabeling in the songbird brain, however the number of cell types and their descriptions are not standardized across the literature using DCX to quantify adult neurogenesis in songbirds. Several studies published since Boseret et al. (2007) have

created new or updated categorization schemes and new nomenclature to classify DCX-stained (DCX+) cells in the songbird brain, making it difficult to compare results across studies. We therefore aimed to create a more standardized classification scheme to describe and quantify types of DCX+ cells in the songbird brain. We created a database of individual DCX+ cell images (n=328) shared with us by various laboratories, comprised of eight species, both males and females, from two brain regions (HVC and hippocampus). We then had observers (n=10) with varying levels of experience and expertise categorize images of these cells into three categories (multipolar, fusiform, and round), using examples and definitions amalgamated from several studies. Overall, 87.5% of cell images could be categorized into one of the three categories with agreement of eight or more observers, the overall inter-rater reliability was 91.8%, and there was no difference in reliability between raters with different experience. Our next step is to repeat the categorization task with experienced and new naïve observers using more accurate definitions. These definitions are based on quantification of a variety of morphological characteristics (e.g., soma shape, number of processes, amount of branching) in cells that were categorized reliably by 9 or 10 observers. Overall, our findings seem to show that our three categories are sufficient to classify DCX+ cells and quantify adult neurogenesis in adult songbirds.

Disclosures: B.M.B. Parks: None. **C.A. Stadnyk:** None. **R.M.W. Gibbs:** None. **S.D.T. Aitken:** None. **A.V. Kingbédé:** None. **S. Moore:** None. **M.F. Guigueno:** None. **L.S. Phillmore:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.01

Topic: F.04. Stress and the Brain

Support: 1F31NS122484-01

Title: Characterization of interneuron glucocorticoid receptor expression in the murine hippocampus

Authors: *A. P. CHORDIA[1], K. L. KRAUS[2], C. L. LASARGE[1], S. C. DANZER[3]; [1]Dept. of Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Med. Scientist Training Program, Univ. of Cincinnati, Cincinnati, OH; [3]Dept. of Anesthesia, Cincinnati Children's Hsptl Med. Cntr, Cincinnati, OH

Abstract: Despite the pervasive impact of stress on psychiatric and neurological disease, much of the circuit- and cell-mechanisms responsible for the limbic regulation of the stress response remain unknown. The hippocampus has become a significant target of stress research in recent

years because of its role in cognitive functioning and neuropathology. Based on preclinical data, the hippocampus is thought to inhibit the stress response and play a crucial part in the hypothalamic pituitary adrenal (HPA) axis. Hippocampal excitatory neurons (dentate granule cells, CA1 and CA3 pyramidal cells) express glucocorticoid receptors (GRs) and can therefore respond directly to serum glucocorticoids. Whether hippocampal interneurons also express GRs, however, is not known. Hippocampal interneurons play a major role in the regulation of neuronal activity, exerting tight inhibitory control over excitatory neurons, and consequently, hippocampal function. Based on the GR expression profiles among interneurons of other limbic brain regions involved in the HPA axis regulation, such as the prefrontal cortex, we hypothesize that hippocampal parvalbumin (PV) and cholecystokinin (CCK) interneurons express GR, whereas somatostatin (SST) interneurons do not. Using immunohistochemistry (IHC), confocal imaging, and Imaris image analysis software, we quantified colocalization for GR, PV and CCK immunolabeling. We confirmed antibody specificity for PV interneurons by also measuring GR immunolabeling in a transgenic mouse line which expresses eGFP among PV interneurons. Both PV and CCK interneurons were found to co-express GRs by immunohistochemistry. Studies are ongoing to determine whether hippocampal SST interneurons express GR. Elucidating the relationship between GR and interneuron populations within the hippocampus is crucial to better understand which cell populations represent direct targets of glucocorticoids and their role in stress-related illness.

Disclosures: A.P. Chordia: None. **K.L. Kraus:** None. **C.L. LaSarge:** None. **S.C. Danzer:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.02

Topic: F.04. Stress and the Brain

Support: NIMH: 5F30 MH117927
NIMH: R37 MH068542
NIMH: R01 MH083862
NYSTEM-C029157
S10 OD018464
HDRF: RGA-13-003
NIA: R01 AG043688

Title: Distinct representations of a novel anxiogenic environment in the ventral hippocampus

Authors: *J. E. BERRY[1], J. C. JIMENEZ[3], S. LIM[1], N. MANFRED[1], K. M. OGBU[1], F. STEFANINI[2], S. FUSI[2], R. HEN[1];

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Abstract: The ability to recognize dangerous situations and environments is crucial for survival, but overestimating risk can lead to pathological avoidance of normal activities, potentially leading to anxiety disorders. Many studies over the past several decades have begun to identify the brain regions underlying threat detection and anxiety behavior. In particular, the ventral hippocampus has emerged as a critical structure for emotional behaviors, including innate anxiety. Recent work from our lab and others has shown that ventral CA1 pyramidal neurons encode information about anxiety, and these CA1 neurons preferentially target downstream structures such as hypothalamus and medial prefrontal cortex. However, the neural representation of anxiogenic environments in the initial stage of the trisynaptic circuit— the dentate gyrus— is unknown. Here, we use Dock10-Cre and Drd2-Cre mouse lines to gain optical access to granule cells and mossy cells, respectively, in the ventral dentate gyrus. Calcium activity was recorded during free exploration of the elevated plus maze (EPM) and open field test (OFT). Single cell activity and population coding were analyzed for mossy cells, granule cells, and CA1 pyramidal neurons. We found that anxiety-related activity was present in granule cells and vCA1, however mossy cells encoded novelty and spatial position. These findings support the notion that different features of an anxiogenic environment are encoded by different cell types, and that anxiety information is present at the earliest stage of the trisynaptic circuit.

Disclosures: J.E. Berry: None. **J.C. Jimenez:** None. **S. Lim:** None. **N. Manfred:** None. **K.M. Ogbu:** None. **F. Stefanini:** None. **S. Fusi:** None. **R. Hen:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.03

Topic: F.04. Stress and the Brain

Support: Hope for Depression Research Foundation

Title: Chromatin footprints in the ventral hippocampus in a double-hit model of heterotypic acute stress

Authors: *S. G. CARADONNA[1], M. R. PAUL[2], K. BULLOCH[3], B. S. MCEWEN[1], J. MARROCCO[1];
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Abstract: Stress induces allostatic responses, whose limits depend on both genetic background and the nature of the challenges. Acute stress generally assembles immediate adaptive responses, although, depending on the intensity of the stressors and individual differences, maladaptive responses may occur and persist through the lifespan and even intersect with later adverse events, as in the case of post-traumatic stress disorder. We investigated affective behavior and genome-wide chromatin accessibility in the ventral hippocampus in a double-hit model of acute stress in adult male and female mice in which heterotypic stressors, acute restraint stress (ARS) and acute swim stress (AS), were applied within a 7-day interval period from each other. Six days after ARS, ARS males showed decreased anxiety-like behavior compared to controls in the light-dark box test, whereas ARS females exhibited no behavioral changes. Using ATAC-sequencing, we then investigated whether there existed sex differences in the epigenetic reprogramming that underlined differences in the behavioral outcome observed one week after ARS and assessed the epigenetic signatures when AS, a second-hit stressor, was applied 7 days after ARS. We found that 1034 gene transcripts were differentially regulated between males and females prior to stress. AS alone induced chromatin reorganization in 3007 gene transcripts in males and 854 transcripts in females 40 minutes after the end of stress, while ARS modulated 2092 transcripts in males and 583 transcripts in females 7 days after stress. When both stressors were combined, the number of differentially regulated transcripts compared to AS or ARS alone was significantly increased in females, which showed 1909 open transcripts, while males showed 2797 modulated transcripts, a number akin to ARS alone. Notably, commonalities in differentially regulated transcripts in AS alone, ARS alone, or with ARS combined with AS accounted for only 0.76% in females and 5.76% in males, indicating that each stressor and the double-hit stress, all induced unique changes in chromatin modification. Together, we found that distinct acute stressors induce allostatic signatures in chromatin reorganization that i) can be observed within minutes after the end of stress, ii) persists one week after the stressor, iii) shows transcripts that are unique to the double-hit acute stress, and iv) differs in males and females.

Disclosures: S.G. Caradonna: None. **M.R. Paul:** None. **K. Bulloch:** None. **B.S. McEwen:** None. **J. Marrocco:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.04

Topic: F.04. Stress and the Brain

Support: VA Grant BX001075
F32MH117913

Title: Distinct forebrain circuits from the subfornical organ regulate fear (IL cortex) or physiological homeostasis (BNST).

Authors: *K. M. J. MCMURRAY[1,2], A. KALATHILL[1], R. SAH[1,2];
[1]Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; [2]DVA, Cincinnati, OH

Abstract: PTSD associates with dysregulated fear/threat responding. Prior fear-related research has focused on responses to externally evoked threats. Yet mounting evidence suggests PTSD patients are highly sensitive to homeostatic threats such as CO_2 inhalation. CO_2 inhalation triggers acidosis and evokes anxiety- and fear-associated defensive behaviors in clinical and preclinical models. Pre-deployment CO_2 sensitivity in veterans associates with later trauma-induced PTSD symptoms, suggesting CO_2 sensitivity pre-dates development of PTSD. Investigating the relationship between homeostatic threats like CO_2 inhalation and externally evoked threats will improve our understanding of PTSD vulnerability. We recently developed a mouse model examining the effect of CO_2-inhalation on later PTSD relevant behaviors and found CO_2 exposure increased later footshock-evoked fear extinction deficits. These effects associated with reduced neuronal activation within the infralimbic (IL) cortex and increased activation in the bed nucleus of the stria terminals (BNST), two regions mediating threat and fear responses. This suggests a convergence of homeostatic CO_2-sensing nodes and forebrain fear nodes, yet the distinct circuits mediating these effects are unknown. We previously reported the subfornical organ (SFO) as a key homeostatic site regulating CO_2-evoked fear. Here, we identified direct SFO projections to both IL and BNST by AAV tract tracing. Using an intersectional chemogenetic (DREADD) approach we tested the hypothesis that inhibiting SFO-IL or SFO-BNST projections during CO_2-inhalation attenuates defensive responding during CO_2-inhalation and delayed CO_2-associated fear extinction deficits. We found inhibiting the SFO-IL, but not SFO-BNST circuit during CO_2 inhalation reduced CO_2-evoked defensive behaviors, and later attenuated CO_2 potentiation of contextual conditioned fear and improved extinction. Given a role of the SFO in maintaining physiological homeostasis via adaptive behaviors like drinking, we determined if inhibiting these circuits affected water consumption. We found inhibition of SFO-BNST, but not SFO-IL projections affected consumption. These data suggest SFO projections have discrete roles in behavioral regulation: the SFO-IL circuit regulates fear associated with homeostatic threat CO_2, while the SFO-BNST circuit regulates adaptive behaviors for homeostatic maintenance. Collectively, these data highlight SFO-IL projections as a key circuit facilitating the convergence of homeostatic threat sensing and long-term fear memory that may contribute to PTSD vulnerability.

Disclosures: K.M.J. McMurray: None. **A. Kalathill:** None. **R. Sah:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.05

Topic: F.04. Stress and the Brain

Support: R01 MH119106

Title: An insular cortex-bed nuclei pathway engages passive avoidance behavior in aversive and non-aversive contexts

Authors: *D. C. HINZ[1], T. D. SKOG[3], R. LINGG[3], J. T. LUNA[2], S. A. ROMIG-MARTIN[1], J. J. RADLEY[3];
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Abstract: For many years the insular cortex (IC) has been appreciated to be a key site for the integration of somatosensory and viscerosensory information. More recent work has demonstrated that in rodents the role of IC extends to promoting adaptive responses to stressful or threatening contexts that favor avoidance or anxiety-like behaviors. However, less consideration has been given to whether IC integrates behavioral with neuroendocrine features under these and in other aversive contexts. Our previous work implicates the anteroventral bed nuclei of the stria terminalis (avBST) in the inhibition of medial prefrontal cortical influences over passive behavioral and hypothalamo-pituitary-adrenal (HPA) responses to stress. Based on these studies, we examined the role of the IC-avBST pathway in HPA and behavioral responses in tests of stress coping and avoidance. Using an intersectional viral expression strategy, the projection from the IC-avBST was transfected with the excitatory DREADD, hM3Dq, or reporter-only virus in rats. Using the shock probe defensive burying test (SPDB) to measure active (escape, defensive burying) and passive (immobility) coping in response to a noxious shock, we found that chemogenetic activation of the IC-avBST pathway (0.1 mg/kg CNO, i.v.) significantly decreased defensive burying (by 51%, $p < 0.05$) without affecting other active or passive behaviors relative to CNO-injected and vehicle-injected controls. Interestingly, although SPDB provoked a reliable increase in HPA output, chemogenetic activation of the IC-avBST pathway failed to modulate this response, as based on radioimmunoassay for corticosterone from blood samples collected over repeated intervals before and after SPDB. Follow-up studies using two additional approach-avoidance tests (elevated plus maze and novelty suppressed feeding test) each revealed that chemogenetic activation of IC-avBST pathway (0.3 mg/kg CNO, i.p.) shifted behavior toward a more passive avoidant phenotype. Taken together, these data show that increasing activity in the IC-avBST pathway promotes a shift toward passive avoidance behavior. The divergent effects of IC-avBST and prefrontal-avBST pathways on passive avoidance suggests a more complex role of avBST in promoting behavioral adaptations to stress than previously envisaged.

Disclosures: D.C. Hinz: None. **T.D. Skog:** None. **R. Lingg:** None. **J.T. Luna:** None. **S.A. Romig-Martin:** None. **J.J. Radley:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.06

Topic: F.04. Stress and the Brain

Support: R01 MH119106

Title: A prefrontal to midbrain periaqueductal gray circuit restrains passive coping in response to stress

Authors: *T. D. SKOG[1], S. B. JOHNSON[2], T. G. BELTZ[3], R. LINGG[3], D. C. HINZ[3], S. A. ROMIG-MARTIN[3], V. VENKATESH[2], N. S. NARAYANAN[5], A. K. JOHNSON[4], J. J. RADLEY[3];
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Abstract: Stress disorders in humans are believed to be due in part to a maladaptive shift in behavior from active toward passive coping strategies as well as increases in glucocorticoid stress hormones and sympathetic tone. While disturbances in medial prefrontal cortex (mPFC) function likely account for this behavioral shift, the basic neurobiological mechanisms underlying this switch are not well understood.To address this question, we employed an intersectional viral optogenetic approach in rats to investigate the role of a mPFC-periaqueductal gray (PAG) pathway in coping behaviors and glucocorticoid stress hormone output. The projection from the rostral prelimbic mPFC to the ventrolateral PAG was transfected with an adeno-associated virus serotype 5 (AAV5) expressing either a Cre-inducible, light-sensitive chloride ion pump *halorhodopsin*, light-sensitive cation channel *channelrhodopsin*, or reporter-only virus. A Cre recombinase-containing, retrogradely transported AAV2 was injected in the ventrolateral PAG to selectively induce opsin expression in PAG-projecting mPFC neurons. Using the shock probe defensive burying test to naturalistically measure active (escape, defensive burying) and passive (immobility) coping behaviors in response to a noxious shock, we found that pathway inactivation given throughout the period of behavioral observation significantly increased the time rats spent immobile (by 65%, $p<.05$) and produced a downward trend in defensive burying of the probe using the bedding material ($p=.07$), while photostimulation attenuated immobility ($p<.05$) without affecting burying. Interestingly, neither manipulation produced a significant effect on blood plasma corticosterone levels before or after shock as tested using radioimmunoassay. As the ventrolateral PAG has been previously implicated in defensive freezing behaviors, our data implicate a novel, long-projection from the mPFC capable of directly inhibiting passive coping in response to stressors. Our results suggest that this diminished activity in this pathway may contribute to understanding behavioral passivity in stress-related psychiatric illnesses.

Disclosures: T.D. Skog: None. **S.B. Johnson:** None. **T.G. Beltz:** None. **R. Lingg:** None. **D.C. Hinz:** None. **S.A. Romig-Martin:** None. **V. Venkatesh:** None. **N.S. Narayanan:** None. **A.K. Johnson:** None. **J.J. Radley:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.07

Topic: F.04. Stress and the Brain

Support: NIH Grant MH123686
Whitehall foundation 2018-12-09

Title: Adult stress exposure does not alter posterior parietal synaptic architecture, sensory processing or working memory.

Authors: *G. LUR, A. Y. FLORES, **S. B. PARK**;
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Abstract: Synapse loss in key brain regions is thought to underlie stress-induced cognitive impairment. Our group has previously shown that repeated exposure to multiple concurrent stressors (RMS, consisting of physical, auditory, and visual stimuli) during adolescence reduces both excitatory and inhibitory synapse density in the mouse posterior parietal cortex (PPC). We linked this synapse loss to deficits in working memory. Whether these findings depended on the specific pre-juvenile timing of RMS exposure (postnatal day 30-40) was unclear. Thus, here we test the effect of the same stress exposure in young adult male mice (postnatal day 70-100). Using immunofluorescence staining, we found no effect of adult RMS on excitatory or inhibitory synapse density in the PPC. Basic behavioral tests (light-dark box and Y-maze) showed no effect of adult RMS on anxiety, motivation, or navigation. A sophisticated, head-fixed behavioral paradigm indicated that adult RMS did not disrupt working memory performance either. Our previous work showed that adolescent RMS specifically disrupted sensory inputs to the PPC. Here, we employed two-photon calcium imaging to assess parietal sensory processing pre- and post-stress. We did not find a notable difference in sensory responsiveness of PPC neurons when comparing adult RMS exposed and control mice. Together, these data suggest that developmental timing critically influences the synaptic, circuit and behavioral outcomes of adverse experience. In our case specifically, stress exposure during the critical period for PPC development - i.e. early adolescence - has a marked effect on parietal circuitry and behavior. In contrast, the adult PPC appears to be robust against relatively mild stress like our RMS paradigm.

Disclosures: G. Lur: None. **A.Y. Flores:** None. **S.B. Park:** None.

Poster

P606. Cortical and Hippocampal Regulation of Stress and Anxiety-Related Behaviors

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P606.08

Topic: F.04. Stress and the Brain

Title: Cortex-wide dynamics of norepinephrine release in resting state

Authors: *D. M. ASHBY[1], A. MCGIRR[2];
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Abstract: Resting state brain activity is characterized by ongoing fluctuations in neuronal activity that have widespread organization at multiple spatial and temporal scales. In mice, genetically encoded calcium sensors are proxies of neural activity, and allow the interrogation of large-scale population activity. At the cellular level this neuronal activity is bidirectionally related to neurotransmitter and neuromodulator release and clearance dynamics. The development of protein biosensors designed to measure specific neurotransmitters and neuromodulators enables the investigation of their dynamics on a sub-second timescale, as well as the relationship of these fluctuations to neuronal calcium activity at the mesoscale. Using combined imaging of the norepinephrine sensor GRAB_NE1m and the red-shifted calcium indicator jrGECO1a, we describe cortical mesoscale dynamics of norepinephrine release in awake head-fixed mice. AAV9-GRAB_NE1m was injected in three bilateral cortical sites in Thy-1 jrGECO1a mice, allowing combined imaging over a large expanse of the dorsal cortical surface (approximately 8x8mm) through a chronic intact skull preparation. After recovery and head-fixation training, GRAB_NE1m and jrGECO1a were simultaneously imaged using a dual camera setup with strobed excitation of GRAB_NE1m (470nm) and jrGECO1a (567nm), in addition to red and green wavelengths for hemodynamic signal correction. In spontaneous resting state, we find evidence for spatial and temporal dynamics in the cortical release of norepinephrine associated with neuronal calcium fluctuations.

Disclosures: D.M. Ashby: None. **A. McGirr:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.01

Topic: F.07. Autonomic Regulation

Support: NIH Grant OT2-OD023867
NIH Grant R21-DK116029
NIH Grant R21-EB024701

Title: Transcutaneous auricular vagus nerve stimulation modulates brainstem functional connectivity and gastric peristalsis in functional dyspepsia

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[1]Martinos Ctr. for Biomed. Imaging, Radiology, [2]Gastroenterology and Ctr. for Neurointestinal Hlth., [3]Cardiovasc. Res. Ctr., Massachusetts Gen. Hosp., Boston, MA; [4]Radiology, Logan Univ., Chesterfield, MT; [5]Korean Inst. of Oriental Med., Daejeon, Korea, Republic of; [6]Med., Harvard Med. Sch., Charlestown, MA

Abstract: Functional dyspepsia (FD) patients experience upper gastrointestinal (GI) symptoms possibly related to gastric motor or sensory dysfunction. The nucleus tractus solitarii (NTS) receives and integrates afference from visceral organs including the GI tract and projects to regions involved in central autonomic regulation and pain processing. This processing of afferent visceral sensation may be modulated by transcutaneous auricular vagus nerve stimulation (taVNS). We hypothesize that altered NTS connectivity to higher brain regions in FD will be associated with motor and sensory dysfunction during a meal challenge, and that tVNS can modulate both the motor function of the gut and gastric mechanosensory processing in the brain. FD (N=12) and healthy control (HC) (N=14) subjects were assessed at two MRI visits, one with active respiratory-gated taVNS and one with sham (no current applied). Subjects consumed their maximum tolerable amount of a 470ml food-based MR-contrast pudding. Post meal, subjects were alternately scanned with stomach MRI and brain fMRI at 3T (3x each). Peristaltic propagation velocity in the antrum was calculated using cross-sectional area time series, as previously published (Sclocco et al. 2021). Average BOLD fMRI timeseries were extracted from a left NTS region and used to generate seed-to-voxel whole-brain functional connectivity maps, which were contrasted between groups and conditions, correcting for multiple comparisons (FSL FLAME1+2, cluster forming threshold $z > 2.3$, FWE cluster size $p < 0.05$). With no taVNS stimulation (sham visit), FDs displayed lower average peristaltic velocity compared to HCs (t-test: HC=5.58 +/- 1.68, FD=3.76 +/- 0.76mm/s, p=0.003). For FD, there was a trend toward significance for higher peristaltic velocity during active taVNS compared to sham (t-test: sham=3.76 +/- 0.76; active=5.03 +/- 1.65mm/s; p=0.09). Additionally, for FD there was a trend toward a significant increase in peristaltic velocity over time, i.e. between the first and last stomach MRI scans for active, but not sham, taVNS visit (t-test: Δsham=-0.02 +/- 2.22, p = 0.98; Δactive=1.24 +/- 2.32mm/s; p=0.08). Relative to HCs, FDs demonstrated increased NTS functional connectivity to pre-supplementary motor area, ventrolateral prefrontal cortex, and left anterior insula when no stimulation was applied. For FD patients, active taVNS decreased NTS connectivity to subgenual anterior cingulate cortex. Our results reveal altered stomach motility and NTS cortical connectivity in FD which are both modulated by active taVNS. Understanding both sensory and motor effects of taVNS will inform applicability of this therapy for FD patients.

Disclosures: H. Fisher: None. **R. Sclocco:** None. **R. Staley:** None. **K. Han:** None. **A. Mendez:** None. **J. Coll-Font:** None. **C. Nguyen:** None. **B. Kuo:** None. **V. Napadow:** F. Consulting Fees (e.g., advisory boards); Cala Health.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.02

Topic: F.07. Autonomic Regulation

Support: NIH grant, R01DK121703
NIH SPARC Program, U18TR002205
NIH SPARC Program, OT2OD030535

Title: Selectivity of abdominal vagus nerve stimulation to evoke changes in emesis and gastrointestinal myoelectric activity in the ferret.

Authors: *C. C. HORN[1], J. E. HARMS[1], M. ZEYDABADINEZHAD[4], S. FULTON[5], M. SCIULLO[5], B. MAHMOUDI[4], L. E. FISHER[2], B. J. YATES[3];
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Abstract: Background: The vagus nerve contains thousands of afferent and efferent fibers for communication between the brain and peripheral organs, including the heart, lungs, stomach, intestine, and pancreas. Vagus nerve stimulation (VNS) is a potential therapy for cardiac, respiratory, gastrointestinal (GI), and metabolic diseases. Cervical VNS has been used to treat epileptic seizures but it is difficult to activate specific pathways with this approach because of the complex mixture of nerve fibers at the cervical level. Here, we placed nerve cuff electrodes on the abdominal vagus nerve to target GI fibers; moreover, we implanted electrodes on the GI serosal surface to record myoelectric responses. Our objective was to determine the VNS parameters producing retching, vomiting, and other adverse behavioral responses for future development of VNS therapy to treat obesity and GI motility disorders while avoiding off-target effects, such as emesis and GI myoelectric dsyrthymia. Methods: All animal experiments were approved by the University of Pittsburgh IACUC. Ferrets were surgically implanted with nerve cuff and GI planar electrodes. Cuffs were placed on the ventral or dorsal abdominal vagus trunks. Planar electrodes were placed on the ventral serosal surface of the stomach at four sites from antrum to fundus and in the proximal and distal duodenum. Following recovery, animals were tested using 10 and 30 Hz VNS on separate days. We used a sweep of parameters on each test day: (1) pulse widths of 0.1 and 0.5 ms; and (2) amplitudes of 0.5, 1, 2, and 3 mA. VNS was applied in combinations of pulse width and amplitude with increasing charge for 10 min for each

trial followed by 10 min of no stimulation. Results: Results show that both short and long pulse widths and both stimulation frequencies produced retching and emesis, with stimulation amplitudes of 1 to 3 mA, depending on the individual animal. There were also dysrythmic myoelectric responses produced by VNS before and after retching and emesis. Conclusions: These results demonstrate the range of VNS parameters sufficient to produce retching, emesis, and dysthymic GI myoelectric responses in the ferret. Future studies will need to confirm these findings using different stimulation sequences and conditions, such as with food intake, to tailor VNS for the treatment of obesity and GI motility disorders.

Disclosures: C.C. Horn: None. **J.E. Harms:** None. **M. Zeydabadinezhad:** None. **S. Fulton:** None. **M. Sciullo:** None. **B. Mahmoudi:** None. **L.E. Fisher:** None. **B.J. Yates:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.03

Topic: F.07. Autonomic Regulation

Support: NIH SPARC OT2OD030538
University of Auckland Doctoral Scholarship

Title: Sparc: modeling and simulating vagal efferent innervation of gastric slow waves

Authors: *O. N. ATHAVALE[1], M. R. DI NATALE[2,3], J. B. FURNESS[2,3], R. AVCI[1], A. R. CLARK[1], Z. LIU[4], L. K. CHENG[1], P. DU[1];
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Abstract: Gastric motility is regulated by neural innervation of gastric smooth muscle by the intrinsic enteric nervous system and indirectly by vagal innervation. However, the relationship between vagal and enteric neural circuits and their influence on gastric electrical and mechanical activity has not been characterized or modeled in detail. This study developed a mathematical model of the stomach incorporating the effects of efferent vagal innervation on gastric slow waves, herein referred to as the “Virtual Stomach”.
The model includes an anatomically-based network for the endings of the gastric branches of vagal efferents, spatial distributions of enteric neurons and enteric neuron axonal projections, based on experimental data from rats and guinea pigs. The current network contained approximately half the realistic number of enteric motor neurons. Neuron spiking simulations were conducted in NEURON 7.8 using a leaky integrate-and-fire artificial cell model, with parameters derived from experimental literature. The results of this simulation were coupled to a

biophysical simulation of gastric slow waves through rule-based changes to biophysical model parameters. The spatiotemporal spiking characteristics were quantified by counting the number of spikes in 1 mm^2 square bins and reported as the mean ± standard deviation across bins. P-values are reported for paired t-tests between inhibitory and excitatory spiking activity. The influence of spiking on the activation of gastric slow waves was investigated qualitatively.
The border between the corpus and fundus regions experienced a greater difference between the number of excitatory and inhibitory spikes than the corpus (1730 ± 655 vs 541 ± 406 spikes s^{-1} mm^{-2}). Inhibitory and excitatory spiking frequency was similar in the corpus (3600 ± 452 vs 3550 ± 564 spikes s^{-1} mm^{-2}; $p = 0.33$), however in the antrum, excitatory spiking frequency was greater (5130 ± 693 vs 4100 ± 838 spikes s^{-1} mm^{-2}; $p < 0.01$). In the gastric smooth muscle model this manifested as multiple sites of slow wave origin, at regional boundaries, with the more frequent distal pacemaker entraining the entire simulation region.
The formation of slow wave origin sites in the slow wave model is likely due to changes in the density of enteric motor neurons across the border region and the different direction of excitatory and inhibitory enteric motor neuron axonal projections. The model proposed here provides a platform for further examination of the effects of neuronal network geometry and stimulation parameters on gastric slow waves through coupling with biophysical multiscale models of gastric electrophysiology and biomechanical contractions.

Disclosures: O.N. Athavale: None. **M.R. Di Natale:** None. **J.B. Furness:** None. **R. Avci:** None. **A.R. Clark:** None. **Z. Liu:** None. **L.K. Cheng:** None. **P. Du:** F. Consulting Fees (e.g., advisory boards); Alimetry Limited.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.04

Topic: F.07. Autonomic Regulation

Support: SPARC Program
NIH Grant OT2025340
NIH Grant R01DK119795
Department of Biomedical Engineering at Duke University

Title: Sparc- comprehensive model of colonic motility

Authors: *B. B. BARTH, W. M. GRILL;
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Abstract: One in five adults in the US suffer from functional gastrointestinal and motility disorders (Talley, 2008). Peripheral nerve stimulation provides a treatment option for patients

whose symptoms are refractory to dietary changes and pharmacological treatment (Payne et al., 2018). Sacral nerve stimulation (SNS) is an FDA-approved therapy for fecal incontinence, but SNS has failed to treat slow-transit constipation in clinical trials despite supporting evidence from animal models (Dinning et al., 2015). The mechanisms of SNS are unknown, and the stimulation parameters or "therapeutic dose" are chosen non-systematically (Carrington et al., 2014). Our objective is to develop a comprehensive model of colonic motility that can enable systematic assessment of the effects SNS parameters on colonic motility (Barth et al., 2017). The computational colonic motility model consists of a spatially-distributed network of biophysically-realistic point cells in NEURON with Python (Hines et al., 2009), and connections constructed in NetPyNE (Dura-Bernal et al., 2019). The cells of the network include neurons, categorized into 9 functional populations and 21 population subsets, enterochromaffin cells, enteric glial cells, 2 populations of smooth muscle fibers, and 4 populations of interstitial cells of Cajal. The 21 subsets are derived from the single-cell atlas of the mouse enteric nervous system (Drokhlyansky et al., 2020). We model the colon as a closed pressure vessel with deformable walls and use Stokes equations to model computational fluid dynamics in the lumen of the colon and pellet propagation. We use particle swarm optimization to calibrate model parameters, including neural mechanosensitive mechanisms, synaptic weights, and the likelihood of connection between two cells of given populations. The model propagates fecal pellets at a velocity proportional to their diameter, consistent with findings in the isolated mouse colon (Heredia et al., 2013). Pellets 1, 1.5, 2, 2.5, and 3 mm in diameter propagate at 0.05, 0.05, 0.21, 0.76, and 0.80 mm / s, compared to target velocities of 0.29, 0.40, 0.55, 0.72, and 0.91 mm / s (Heredia et al., 2013). The comprehensive model of colonic motility provides a platform to examine pathological mechanisms and explore novel therapeutics including modeling pharmacological effects and electrical stimulation.

Disclosures: B.B. Barth: None. **W.M. Grill:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.05

Topic: F.07. Autonomic Regulation

Support: NIH Grant SPARC Program OD023847
NIH Grant SPARC Program OD030538

Title: SPARC: Neurons in the nucleus tractus solitarius respond to gastric electric stimulation with orientation selectivity

Authors: *J. CAO[1], X. WANG[1], T. L. POWLEY[2], Z. LIU[3,1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Purdue Univ., W Lafayette, IN; [3]Purdue Univ., West Lafayette, IN

Abstract: Background: The nucleus tractus solitarius (NTS) in the brainstem receives sensory information from the stomach primarily relayed through the vagus. The vagal afferent nerves innervate the stomach with structurally and functionally specialized receptors. As one type of sensory receptors, intramuscular arrays (IMAs) align themselves with circular and longitudinal muscle fibers and presumably encode and transmit muscle activity to the NTS as well as its downstream nuclei. In this study, we aimed to characterize the functional specialization of gastric neurons in NTS, by evaluating their responses to selective activation of circular and longitudinal IMAs given gastric electrical stimulation (GES) in different orientations and amplitudes. **Methods:** We designed the stimulation paradigm according to the distribution and layout of IMAs in the rat stomach. Two stimulating targets were specified in the distal forestomach. One was on the greater curvature and with dense longitudinal IMAs; the other was on the ventral stomach and with dense circular IMAs. To either location, GES was applied with different electrode pairs, generating short (0.3ms) and weak (0.6 to 1mA) current stimuli in four possible directions. During GES, multi-unit activity (MUA) in NTS was recorded with a 32-channel silicon probe and then decomposed into single-unit activity (SUA) by spike sorting. For both SUA and MUA, the post-stimulus firing rate and peri-stimulus time histogram (PSTH) were evaluated as a function of current orientation and stimulation targets of GES in seven rats. **Results:** Both multi and single-unit responses were highly selective to the orientation of GES. The largest response occurred when the current flowed in the same direction as IMAs. For GES targeting longitudinal IMAs, the preferred orientation was longitudinal, pointing from distal to proximal stomach. For GES targeting circular IMAs, the preferred orientation was circular, pointing from the less curvature to the greater curvature. This orientation selectivity was also consistent across different stimulus amplitudes (0.6, 0.8, and 1 mA). **Conclusion:** Our results suggest that gastric neurons in NTS may encode the orientation-specific activity of the stomach relayed by the ascending vagal pathway. This finding indicates that the orientation of GES is critical to the effective engagement of vagal afferents and should be considered in light of the structural phenotypes of vagal terminals in the stomach.

Disclosures: J. Cao: None. **X. Wang:** None. **T.L. Powley:** None. **Z. Liu:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.06

Topic: F.07. Autonomic Regulation

Support: NIH Grant 1OT2OD030535-01

Title: Sparc: gastric emptying of liquids initiated by neural stimuli -- a compartmental modeling approach

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[1]Dept. of Chem. and Biomolecular Engin., Lehigh Univ., Bethlehem, PA; [2]Emory Univ., Atlanta, GA

Abstract: Introduction The stomach in the Gastrointestinal (GI) tract is responsible for the storage, breakdown, and transport of food to the duodenum for digestion. Previously, finite element methods and computational fluid dynamics (CFD) techniques have been reported that model this complex function involving interacting neural and smooth muscle cell (SMC) activity, deformable wall boundaries and the resulting pulsating flow. Invariably, these models are computationally expensive. We use a compartmental modeling approach that is computationally cheaper and suitable for designing model-based closed-loop controllers as neurostimulation-based therapy for treating GI disorders.**Methods** The stomach is divided into seven compartments: Fundus, Upper Corpus (pacemaker), Lower Corpus, Proximal/Middle/Terminal Antrum and Pyloric sphincter. Each compartment is modeled with a set of ordinary differential equations with respect to time. The stomach is stimulated at the pacemaker region which results in slow waves in the SMC. A simplified set of equations captures the electro-chemo-mechanical coupling of smooth muscle contractions. The muscle wall of the stomach is modeled as a hyper-viscoelastic material. The muscle contractions give rise to peristalsis in the gastric walls which influences the velocity of gastric fluid flow resulting in emptying of stomach. The velocity profile for peristalsis is modeled using Shapiro’s model. The fundus acts as a pressure bubble which also influences gastric emptying. The equation for the pressure bubble is derived from Bernoulli's equation for fluids. The time taken for gastric emptying depends on the volume of gastric contents in the stomach, the nutrient content, and the viscosity. When chyme from the pyloric sphincter enters the duodenum, a feedback signal may be sent to the pyloric sphincter to inhibit the chyme flow to the duodenum to prevent overloading if the liquid is high in calorie content. This feedback signal is also incorporated in the model. The resulting model is solved using differential equation solvers in MATLAB.**Results/Conclusions** The model computes membrane voltage from SMC, intracellular calcium concentration, stress and stretch values during the SMC contraction, fluid velocity and volume of the stomach during gastric emptying. These results are then compared and validated against other simulations: (1) the CFD study of liquid gastric emptying (Li, Gin (2020)); (2) FEM viscoelastic SMC model (Panda, Buist (2021)); (3) experimental data from Vella, Camilleri (2017) on mechanical and hormonal response to nutrient ingestion.

Disclosures: S. Fernandes: None. **M.V. Kothare:** None. **B. Mahmoudi:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.07

Topic: F.07. Autonomic Regulation

Support: NHLBI U01 HL133360
NIH/OD OT2 OD030534

Title: SPARC: a 3D anatomical and molecular map of cardiac vagal motoneurons

Authors: *E. HORNUNG, S. ROBBINS, S. ACHANTA, A. SRIVASTAVA, A. MOSS, J. SCHWABER, R. VADIGEPALLI;
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Abstract: The pioneering work of many in the single cell transcriptomics field has begun to illuminate the diversity of cell types in the brain. However, cardiac-projecting vagal motoneurons of the Dorsal Motor Nucleus of the Vagus (DMV) are relatively understudied. By combining principles of neuroscience and single cell transcriptomics, we begin to explore the transcriptomic phenotypes of cardiac-projecting DMV neurons. Neuromodulatory co-transmission is an emerging discipline of neuroscience, and the work of the Allen Institute suggests transcriptomics of neuropeptides and corresponding receptors may characterize cell types. We find that DMV neurons are not solely cholinergic, but simultaneously catecholaminergic and GABAergic, including those that project to the heart. These findings challenge the conventional neuronal nomenclature. We propose inputs determine phenotype, and work done on the Mouse Brain Atlas supports this premise, suggesting a transcriptomic diversification, less region-specific, results from interaction with the environment. We present here a characterization of DMV cell types by input-output transcriptomics. Remote ischemic preconditioning (RIPC) is an invasive cardioprotective physiological intervention. The literature shows the DMV is required for the cardioprotective effect of RIPC. We combine high throughput transcriptomics, 3D anatomical mapping, and neural tracing in 12 week old Sprague Dawley male and female rats to interrogate the phenotypes of DMV neurons. To distinguish potential mechanisms of cardioprotection, we highlight the functional significance of several transcripts for secreted proteins in cardiac-projecting DMV neurons. We have spatially mapped the location and molecular phenotypes of these single cells in the DMV and Nucleus Ambiguus (NA). The cardiac-projecting neurons of the DMV extend approximately 2.5 mm rostrocaudally. The cardiac-projecting neurons of the left DMV are particularly prominent caudally, while the cardiac-projecting neurons of the right DMV are particularly prominent rostrally. A unique phenotype, with transcriptomic markers of strong cardioprotective potential, is present at the rostrocaudal position of left DMV previously shown to affect ventricular contractility. There is consistency between our findings from three spatial transcriptomics techniques. Based on this, we propose a 3D anatomical and molecular map of cardiac vagal motoneurons.

Disclosures: E. Hornung: None. **S. Robbins:** None. **S. Achanta:** None. **A. Srivastava:** None. **A. Moss:** None. **J. Schwaber:** None. **R. Vadigepalli:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.08

Topic: F.07. Autonomic Regulation

Support: T32 OD011126
R01 HL128454
R01 HL132836

Title: Unilateral vagotomy in rats blunts respiratory responses to vagal afferent stimulation

Authors: *G. C. HOFMANN[1], D. D. KLINE[2], E. M. HASSER[2];
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Abstract: The nucleus tractus solitarii (nTS) of the brainstem is the initial site of integration of sensory input from the cardiorespiratory system, including afferents from the vagus nerve carrying sensory input from the heart, lungs, and gut. The nTS is an important site of neuroplasticity in health and disease. Such plasticity is dependent upon a variety of factors, including neuronal and astrocytic activity, with the latter regulating glutamate signaling. We have shown that unilateral transection of the vagus nerve to decrease afferent input to the nTS results in morphologic changes in brainstem glia, including augmented astrocyte branching. Vagal nerve transection also decreases nTS synaptic transmission studied in vitro. The influence of vagotomy on in vivo cardiorespiratory function via afferent-driven glutamatergic signaling and astrocytes is unknown. We hypothesized that chronic vagotomy blunts cardiorespiratory responses to vagal afferent stimulation via altered glutamate signaling. Male Sprague-Dawley rats (6-wk, N=13) were randomly assigned to vagotomy, sham (surgery without nerve transection), or control (no surgery) groups. Right cervical vagus nerve transection caudal to the nodose ganglion was performed. One week after surgery, rats were anesthetized with Inactin, ventilated, and instrumented to measure heart rate (HR), blood pressure (BP), splanchnic sympathetic and phrenic nerve (SSNA and PhrNA, respectively) activity, and for cervical vagal nerve stimulation. Intact vagus nerves were transected acutely to prevent efferent stimulation. Left and right vagus nerves were stimulated (20 sec, 0.2mA, 10Hz, 0.5ms pulse width) and responses recorded for 3 minutes. Vagal nerve stimulation in sham and control groups decreased PhrNA frequency and amplitude (apnea). However, unilateral vagotomy blunted the decrease in PhrNA ($p<0.05$) due to stimulation of the severed (right) vagus compared to right-sided stimulation of controls and shams, and compared to left-sided (intact) stimulation. Increases in HR, BP, and SSNA to stimulation were similar among groups as well as between their ipsi- and contralateral sides. Preliminary experiments in which glutamate was nanoinjected into the right

nTS showed a trend towards blunted BP and HR responses after vagotomy compared to shams. The blunted responses to both physiological glutamate release (stimulation) and exogenous glutamate administration may indicate that vagotomy alters glutamate activity via postsynaptic or astrocytic mechanisms. Additional experiments are needed to determine the mechanisms of altered nTS response to changes in afferent input in cardiorespiratory health and disease.

Disclosures: G.C. Hofmann: None. **D.D. Kline:** None. **E.M. Hasser:** None.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.09

Topic: F.07. Autonomic Regulation

Support: NIH Grant R01-HL-141560
AHA Grant 275076
LivaNova USA

Title: Vagus nerve stimulation mitigates cardiac hypertrophic remodeling in rats with pressure overload

Authors: T. BANKS[1], **A. FARRAND**[1], C. COOPER[1], M. HERNDON[1], A. HEBERT[1], B. KENKNIGHT[2], *E. BEAUMONT[1];
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Abstract: Vagus Nerve Stimulation (VNS) is currently being investigated in the clinic for treatment of heart failure. Cardiac dysfunction in heart failure with reduced ejection fraction (HFrEF) is thought to be due in part to a chronic neuroinflammatory state as measured by increased pro-inflammatory cytokines in key autonomic brain nuclei. While the primary mechanism of action for VNS remains unknown, VNS exhibits anti-inflammatory properties ranging from reduced activation of glia and macrophages to a reduction of pro-inflammatory cytokine production both in the periphery and in the central nervous system. Therefore, the purpose of this study is to provide insight into VNS treatment for HFrEF via a pressure overload (PO) rat model. The PO was induced by constricting the aorta ~30-50% under anesthesia. Following the PO procedure, half of the rats underwent surgical implantation of VNS devices with a bipolar lead connected to the left cervical vagus nerve. Rats were separated into control (n=8), PO (n=6), and PO + VNS (n=12) treatment groups. We initiated VNS therapy for 2 months upon reaching a 20% decrease in left ventricular relative ejection fraction (EF, p=0.0031 compared to baseline). These PO + VNS rats were compared to age matched PO and Controls. Regular echocardiography, electrocardiogram readings and weights were recorded at baseline

and at regular intervals throughout the study for all rats. Brains were preserved and sectioned for immunohistochemical staining, and hearts were removed and weighed at termination for a final heart to body weight ratio measurement. Our findings thus far indicate that while PO reduced EF ~40% at termination (p=0.0348 compared to study midpoint, p=0.0179 compared to baseline), VNS treatment resulted in a restoration of EF back to control levels (p<0.0001 compared to study midpoint, no significant difference compared to baseline). Further, the heart/body weight ratio increased for PO rats compared to control (p=0.0386) and PO + VNS rats (p=0.0319). This demonstrates that VNS therapy mitigates physiological markers of heart failure. To assess potential mechanisms of action, neuroinflammatory markers including glial density and activation are being examined in key nuclei relevant to heart failure including both the nucleus of the solitary tract and the paraventricular nucleus of the hypothalamus. These changes will provide insight into the role of neuroinflammatory mechanisms for VNS effects in this PO model.

Disclosures: T. Banks: None. **A. Farrand:** None. **C. Cooper:** None. **M. Herndon:** None. **A. Hebert:** None. **B. KenKnight:** A. Employment/Salary (full or part-time):; LivaNova USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LivaNova USA shareholder. **E. Beaumont:** 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.; LivaNova USA funding to independently conduct study. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); LivaNova USA devices for VNS.

Poster

P628. Gastric, Intestinal, and Vagal Regulation

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P628.10

Topic: F.07. Autonomic Regulation

Support: NIH Grant OD023847
NIH Grant OD030538

Title: Sparc: gastric myoelectrical activity is intrinsically synchronized with fmri activity in the rat brain

Authors: J. CAO[1], X. WANG[2], U. SCHEVEN[2], ***Z. LIU**[2];
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Abstract: SPARC: Gastric Myoelectrical Activity isIntrinsically Synchronized with fMRI Activity in the rat Brain Jiayue Cao[1], Xiaokai Wang[1], Ulrich scheven[2], and Zhongming Liu[13*]

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Background: Stomach-brain interactions are essential to interoceptive feeling and can influence cognition andemotion. However, the central neural circuits underlying stomach-brain interactions are poorlyunderstood. Recent studies tackle this problem and use simultaneous recordings of functionalmagnetic resonance imaging (fMRI)and electrogastrogram to report the phase-coupling betweenbrain activity and gastric pace-making activity. In this study, we investigated the stomach-braininteraction using a more direct measurement from the stomach, the gastric myoelectrical activity(GMA),and mapped brain regions with fMRI signals coupled to both phase changes and powerfluctuations of GMA.
Method: We implanted two pairs of electrodes on the medial (corpus)and distal (antrum)stomach torecord GMA in four rats. Each rat underwent two fMRI experiments simultaneously with GMArecording in the fasting and postprandial states. The relationship between GMA and fMRIsignals was assessed in terms of both phase and power coupling. The phase-coupling wascalculated as the coherence between fMRI and GMA at the gastric pace-making frequency; thepower-coupling was evaluated as the correlation between fMRI and GMA power fluctuations.
Results: This study confirmed the phase-coupling between the stomach and the brain in rats in bothfasting and postprandial states. The phase-coupling occurred in a broad set of brain regions,including the somatomotor cortex, visual cortex, and basal ganglia. The coupling was strongerwith gastric activity from the corpus than the antrum. In addition, the power fluctuations ofGMA were also correlated with fMRI activity in the brain, such as the anterior cingulate cortex,insular cortex, and somatomotor cortex. The power correlation was stronger in the postprandialstate than the fasting state and with the corpus than the antrum.
Conclusion: This study confirms that brain fMRI activity is synchronized with both the phase and power ofgastric myoelectric activity, more so for the corpus than the antrum. The results suggest that thebrain monitors the stomach for both gastric rhythms and contractional power and may contain atopographic mapping of the stomach function.

Disclosures: J. Cao: None. **X. Wang:** None. **U. Scheven:** None. **Z. Liu:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.01

Topic: G.05. Mood Disorders

Title: Proliferation of putative GABA/glutamate co-releasing terminals in the primate habenula

Authors: *L. RIOS[1], C. TAN[2], A. HAJEISSA[1], V. NARLA[1], K. GLEASON[1], C. A. TAMMINGA[3], S. SHABEL[1];
[2]Psychiatry, [1]UT Southwestern Med. Ctr., Dallas, TX; [3]Univ. of Texas Southwestern Med. Ctr. at Dallas, Dallas, TX

Abstract: The lateral habenula (LHb) responds to aversive stimuli, and its hyperactivity is hypothesized to contribute to depression. Most of the central nervous system uses a balance of excitatory and inhibitory transmission from separate inputs to control neural activity. However, multiple inputs to the lateral habenula co-release GABA with glutamate in rodents; the balance of GABA and glutamate at these synapses may regulate LHb responses to aversive stimuli and mood. Here, we investigated whether the magnitude and topography of co-release of GABA and glutamate in the LHb is conserved in primates. Our data indicate substantially increased co-labeling of GAD (the synthesizing enzyme for GABA) and VGLUT2 (vesicular glutamate transporter), as well as VGAT (vesicular GABA transporter) and VGLUT2, in synaptic terminals in the monkey and human LHb, consistent with increased co-release of GABA and glutamate from individual terminals onto primate LHb neurons. We also found an expanded topography of co-release in the primate LHb, perhaps partly due to expansion of the LHb in primates. Thus, co-release of GABA with glutamate may be a mechanism for regulation of LHb activity and mood that proliferated during primate evolution.

Disclosures: L. Rios: None. **C. Tan:** None. **A. Hajeissa:** None. **V. Narla:** None. **K. Gleason:** None. **C.A. Tamminga:** None. **S. Shabel:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.02

Topic: G.05. Mood Disorders

Support: CNPq (HMTB 1B Researcher Grant)
CAPES
FAPERGS

Title: Looking at both sides: a scoping review of neurochemical asymmetries in animal models of depression

Authors: *F. B. ALMEIDA[1,2], R. L. FIORE[2], R. GOMEZ[4], M. S. NIN[1,4,5], H. M. T. BARROS[1,2,3];
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Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; [5]Dept. de Farmacologia, Univ. Federal do Rio Grande (FURG), Rio Grande, Brazil

Abstract: Many clinical studies have demonstrated an asymmetry in cortical function of depressed individuals, contrasting with a symmetrical activity observed in non-depressed controls. However, the relationship of this functional asymmetry with the neurochemistry that drives the underlying mechanisms of depression is not clear. In this sense, preclinical studies that investigate the hemisphere-specific role of neurochemical markers in depressive-like behaviors and antidepressant action could shed light on this matter. Thus, we conducted a systematic scoping review to map the preclinical literature on depression that investigated hemisphere-specific levels of neurochemical markers related to animal models of depression and antidepressant treatment. We searched four different databases (PubMed, EMBASE, PsycInfo and LILACS) for relevant papers that were subsequently screened for eligibility, and a final number of 23 articles were included. Our results show that animal models of depression used were based on stress (either prenatal, on early-life or on adulthood), congenitally depressed rat lines, or were pharmacologically induced. Several different antidepressants were used as pharmacotherapies, but other classes of substances such as neurosteroids and hypertensives were also administered. The array of neurochemical markers assessed was highly heterogeneous, with dopamine, serotonin, brain-derived neurotrophic factor (BDNF) and subunits of the $GABA_A$ receptor appearing with the higher frequencies. For each neurochemical marker, studies were of significantly different experimental designs to derive generalizable conclusions, but experiments in which laterality was analyzed as a factor in ANOVA statistics were mapped and quantified. Approximately one third (32%) of all experiments that were analyzed this way showed an interaction with the laterality factor, demonstrating a significant impact of hemisphere for the measurement of neurochemical markers. Our results underscore the importance of considering cerebral laterality when measuring neurochemical markers relevant for depression, while pointing to potential issues with the design of neurochemical analysis and with publication/reporting bias in the field. More research of this kind is needed to allow for cross-study comparison of inter-hemispheric neurochemical data in animal models of depression.

Disclosures: F.B. Almeida: None. **R.L. Fiore:** None. **R. Gomez:** None. **M.S. Nin:** None. **H.M.T. Barros:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.03

Topic: G.05. Mood Disorders

Support: NIH Grant MH081917

AHA Postdoctoral Fellowship

Title: Activation of a novel α_{2A}AR-spinophilin-cofilin axis determines the effect of α_2 adrenergic drugs on fear memory reconsolidation

Authors: S. SAGGU[1], Y. CHEN[1], H. WANG[1], C. COTTINGHAM[1], S. ZHANG[2], C. AUGELLI-SZAFRAN[2], K. JIAO[1], X.-Y. LU[3], *Q. WANG[1];
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Abstract: Posttraumatic stress disorder (PTSD) after the pandemic has emerged as a major neuropsychiatric component of post-acute COVID-19 syndrome, yet the current pharmacotherapy for PTSD is limited. The use of adrenergic drugs to treat PTSD has been suggested; however, it is hindered by conflicting clinical results and a lack of mechanistic understanding of drug actions. Our studies, using both genetically modified mice and human induced pluripotent stem cell-derived neurons, reveal a novel α_{2A} adrenergic receptor (α_{2A}AR)-spinophilin-cofilin axis in the hippocampus that is critical for regulation of contextual fear memory reconsolidation. In addition, we have found that two α_2 ligands, clonidine and guanfacine, exhibit differential abilities in activating this signaling axis to disrupt fear memory reconsolidation. Stimulation of α_{2A}AR with clonidine, but not guanfacine, promotes the interaction of cofilin, an actin binding protein, with the dendritic spine scaffolding protein spinophilin to induce cofilin activation at the synapse. Spinophilin-dependent regulation of cofilin is required for clonidine-induced disruption of contextual fear memory reconsolidation. Our results inform interpretation of differential clinical observations of these two drugs on PTSD, and suggest that clonidine could provide immediate treatment for PTSD symptoms related to the current pandemic. Furthermore, our study indicates that modulation of dendritic spine morphology may represent an effective strategy for the development of new pharmacotherapies for PTSD.

Disclosures: S. Saggu: None. **Y. Chen:** None. **H. Wang:** None. **C. Cottingham:** None. **S. Zhang:** None. **C. Augelli-Szafran:** None. **K. Jiao:** None. **X. Lu:** None. **Q. Wang:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.04

Topic: G.05. Mood Disorders

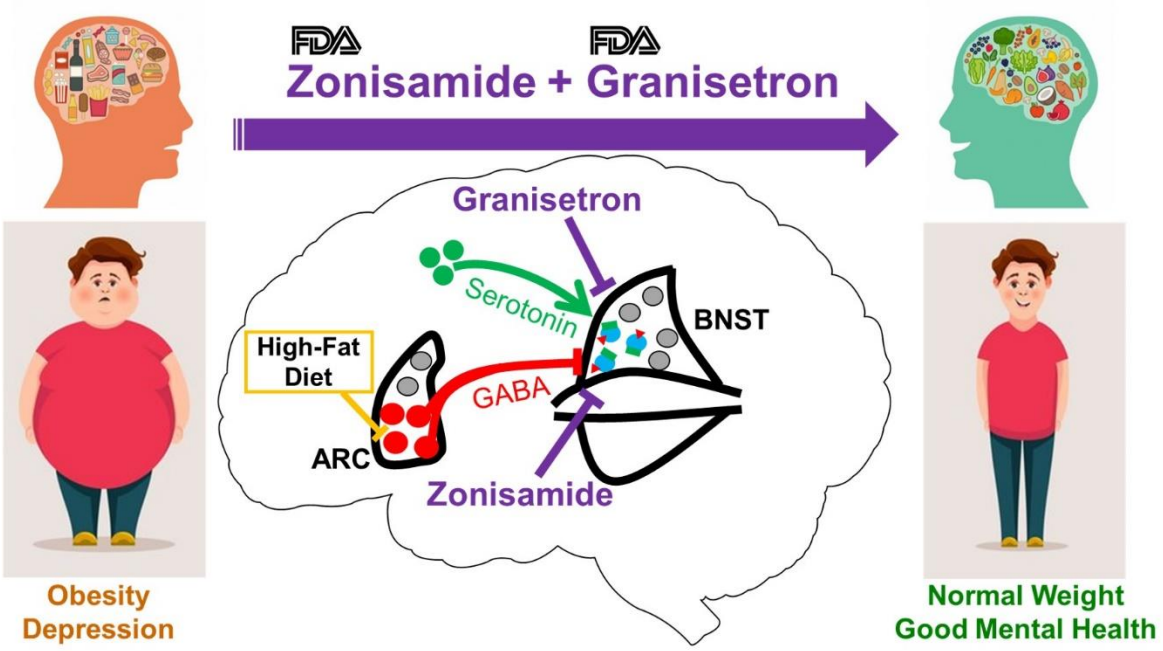
Support: NIH Grant 1R01DK109194
NIH Grant 1R56DK109194
Pew Charitable Trust

USDA/CRIS grants 3092-5-001-059
ADA Grants 7-13-JF-61
ADA Grants 1-17-PDF-138
NIH COBRE grant P20 GM135002

Title: Reciprocal control of obesity and anxiety-depressive disorder via a GABA and serotonin neural circuit

Authors: *Q. WU[1], G. XIA[2], Y. HAN[2], F. MENG[3], Y. HE[1], D. SRISAI[4], R. D. PALMITER[5], Y. XU[1];
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Abstract: The high comorbidity between obesity and mental disorders, such as depression and anxiety, often exacerbates metabolic and neurological symptoms significantly. However, neural mechanisms that underlie reciprocal control of feeding and mental states are largely elusive. Here we report that melanocortin 4 receptor (MC4R) neurons located in the dorsal bed nucleus of the stria terminus (dBNST) engage in the regulation of mentally associated weight gain by receiving GABAergic projections from hypothalamic AgRP neurons onto α5-containing $GABA_A$ receptors and serotonergic afferents onto 5-HT_3 receptors. Chronic treatment with a high-fat diet (HFD) significantly blunts the hyperexcitability of AgRP neurons in response to not only hunger but also anxiety and depression-like stimuli. Such HFD-mediated desensitization reduces GABAergic outputs from AgRP neurons to downstream $MC4R^{dBNST}$ neurons, resulting into severe mental dysregulation. Genetic enhancement of the $GABA_AR$-α5 or suppression of the 5-HT_3R within the $MC4R^{dBNST}$ neurons not only abolishes HFD-induced anxiety and depression, but also robustly reduces body weight by suppression of food intake. To gain further translational insights, we revealed that combined treatment of zonisamide (enhancing the $GABA_AR$-α5 signaling) and granisetron (a selective 5-HT_3R antagonist) alleviates mental dysfunction and yields a robust reversal of diet-induced obesity by reducing total calorie intake and altering food preference towards a healthy low-fat diet. Our results unveil a neural mechanism for a reciprocal control of appetite and mental states, which culminates in a novel zonisamide-granisetron cocktail therapy for potential tackling the psychosis-obesity comorbidity.



Disclosures: Q. Wu: None. **G. Xia:** None. **Y. Han:** None. **F. Meng:** None. **Y. He:** None. **D. Srisai:** None. **R.D. Palmiter:** None. **Y. Xu:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.05

Topic: G.05. Mood Disorders

Title: Acute stress reduces effort-related motivation in male and female rodents

Authors: *E. ERRANTE, O. I. AKINBO, L. MATUSZEWICH;
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Abstract: Motivational dysfunction is present in a variety of disorders, including major depressive disorder, schizophrenia, and Parkinsonism (Demyttenaere et al., 2005) and can negatively impact social functioning, employment, and treatment response (Tylee et al., 1999). Outside stressors and stress hormones have been shown to contribute to depressive episodes in humans and depressive-like behaviors in rodents, including reducing motivated behaviors (Hammen, 2005; Sinha et al., 2018). One approach of examining motivation in both humans and animals is to assess effort-related decision making procedures, as the subject is given a choice between high effort/reward and low effort/reward options (Errante et al., 2021; Shafiei et al., 2012; Treadway et al., 2012). Despite prior research consistently showing sex differences in the

prevalence of depression and depressive-like behaviors in humans, little research has examined the effects of stress on effort-related decision making behaviors in female rats. Thus, the current study investigated the impact of acute stressors or the stress peptide hormone CRF on effort-related choice procedures in both sexes. Male and female rodents were trained on a fixed ratio 5/chow choice feeding procedure for a highly valued sucrose pellet reward or to approach and consume freely available lab chow in an operant chamber. Once responding was stable, rats experienced an acute stressor (Group 1: restraint stress, yohimbine, and food restriction) or a systemic manipulation of CRF (Group 2: CRF peptide, restraint stress, and CRF antagonist antalarmin with restraint stress). Baseline performance in the effort-related decision making task was similar across both sexes ($p > 0.05$). Exposure to restraint stress for 1 hour or yohimbine in group 1 resulted in lower levels of lever pressing in both male and female rodents ($p < 0.05$). In group 2, restraint stress also resulted in lower levels of lever pressing in both male and female rodents ($p < 0.05$); however, antalarmin did not attenuate this response. Further, CRF did not negatively alter performance. Collectively, the current project suggests that stress negatively alters
motivation in both male and female rodents equally; however, more work needs to be done in order to fully understand this phenomenon to improve treatment for mental illnesses associated with motivation.

Disclosures: E. Errante: None. **O.I. Akinbo:** None. **L. Matuszewich:** None.

Poster

P709. Neuro-Molecular Mechanisms of Relevance to Psychiatric Conditions

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P709.06

Topic: G.05. Mood Disorders

Title: Leveraging evolution to unravel the neuro-molecular basis of psychiatric disorders in the human amygdala

Authors: *J. HAN[1,2], H. A. HOFMANN[2,1,3];
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Abstract: The toll of psychiatric disorders is staggering, with an estimated 51.5 million adult sufferers in the US alone! The development of effective interventions has been hampered by the fact that the underlying molecular mechanisms are still not well understood. Because the complex phenotypes of human mood disorders, in particular, cannot be readily recapitulated in non-human animals, any animal model of a psychiatric disorder is necessarily limited in its applicability to the human condition. Nevertheless, numerous studies have shown that abnormalities of the subpallial amygdala - an evolutionarily ancient forebrain region critical to

decision-making and emotional responses - are associated with psychiatric disorders. However, this research cannot explain why certain gene expression modules are susceptible to dysregulation in specific mood disorders or how unique these processes might be to humans. We hypothesized that gene expression modules dysregulated in psychiatric disorders have been gained in the lineage leading to humans. We used phylogenetic comparative transcriptome analyses to understand how evolution has shaped neuromolecular pathways in the (medial) amygdala that may leave us vulnerable to psychiatric disorders. We first collected and normalized publicly available transcriptome datasets of (medial) amygdala (n=351 individuals) for 12 vertebrate species, representing mammals, birds, reptiles, and teleosts. Next, we reconstructed ancestral amygdala transcriptomes for vertebrates, amniotes, mammals, and primates, which allowed us to identify evolutionary conserved "core transcriptomes" of the medial amygdala and its putative non-mammalian homologs. We found that <2% of the genes expressed in the human amygdala transcriptome have been gained compared with the ancestral transcriptome of the common ancestor of primates, suggesting that most dysregulated in psychiatric disorders are not human-specific. We then identified the gene co-expression modules that are either evolutionarily conserved or have evolved *de novo* in the lineage leading to humans and again found that most of these modules are evolutionarily ancient. Finally, few gene co-expression modules dysregulated in different psychiatric disorders are uniquely human and instead display deep evolutionary roots. Our novel approach illuminates the surprising evolutionary origins of the molecular mechanisms underlying complex (social) behavior and their vulnerability to dysregulation in various psychiatric disorders.

Disclosures: J. Han: None. **H.A. Hofmann:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.01

Topic: G.09. Drugs of Abuse and Addiction

Support: F31DA047068
R01DA006214

Title: Auditory cocaine-cue orienting behavior in rats: role of locus coeruleus

Authors: *M. A. PRESKER[1], S. A. TOMBOKAN[1], G. S. ASTON-JONES[2];
[1]Rutgers - The State Univ. of New Jersey, Piscataway, NJ; [2]Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Enhanced behavioral reactivity to and altered neural encoding of drug-associated cues is a primary feature of addiction that contributes to relapse, a primary therapeutic target.

Plasticity in attention and sensory systems may underlie altered drug-cue reactivity in cocaine (COC) addiction. The locus coeruleus (LC) is a noradrenergic brainstem nucleus that projects widely throughout the neocortex and modulates attention and sensation. Although LC is important for drug-cue memories and stress-induced drug seeking, it is not clear what role LC plays in encoding drug-cue salience. We developed a behavioral paradigm to examine the effect of an auditory COC cue on a natural reward operant behavior (nosepoking for water). Rats learned to self-administer water and then underwent classical conditioning to associate one tone with COC (20mg/kg, ip) and another with saline. After conditioning, the effect of the tone on responding for water and stimulus orienting was measured. Behavior was recorded by operant chambers equipped with photodiodes and video cameras. Preliminary results indicate that the COC tone exerted significantly different behavioral control over both responding for water and stimulus orienting compared to the saline tone. This behavioral activation correlates with a significant increase in LC activity measured by Fos expression. Furthermore, the COC tone functioned as a conditioned reinforcer and supported the acquisition of a novel operant response (lever press). Ongoing experiments employ chemogenetic inhibition to test the role of LC in the ACQUISITION (conditioning) and EXPRESSION (tone testing) of the drug-tone association, respectively. TH-cre+ rats (N=36) received virus injections to express the inhibitory hM4Di DREADD receptor or GFP in LC (n=18 each). Next, rats trained on the water task and were split into ACQUISITION and EXPRESSION groups, counterbalanced by virus and behavior. ACQUISITION rats were conditioned and tested as described, except they were pre-treated with CNO (1mg/kg, ip) to inhibit LC during COC tone conditioning. EXPRESSION rats were conditioned normally except they were pretreated with CNO prior to tone testing sessions. All behavior was analyzed between subjects, to determine effects of LC inhibition by hM4Di, and within subjects, to determine cue specificity. Preliminary findings indicate that a COC cue can control attention and natural reward motivated behavior, and that LC encodes aspects of these responses. Ongoing experiments will test the causal role of LC in the acquisition and expression of this response, potentially indicating a new avenue for addiction treatment.

Disclosures: M.A. Presker: None. **S.A. Tombokan:** None. **G.S. Aston-Jones:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant K12 GM093854
NIH Grant K99DA045765
NIH Grant R01 DA006214

Title: The orexin/hypocretin system drives affective behaviors associated with acute abstience from oxycodone.

Authors: *J. E. FRAGALE, V. A. STIRITZ, K. A. NEWMAN, M. H. JAMES, G. S. ASTON-JONES;
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Abstract: Opioid use disorder (OUD) is a chronic and relapsing disorder that has become a national emergency.Misuse of prescription opioids, like oxycodone, is a major contributing factor to the current crisis.Although the rewarding properties of prescription opioids are important for initial drug use, aversivewithdrawal states are believed to better explain the compulsive nature of addiction. Orexins (hypocretins)are neuropeptides produced in the hypothalamus that are important for physical withdrawal; however,less is known regarding the role of orexins in affective withdrawal symptoms. Here, we establish an animalmodel of negative affect associated with acute abstinence from oxycodone, and investigate thecontribution of the orexin system. Male Sprague Dawley rats were treated with non-contingentoxycodone (n=16) or saline (n=16) for 21 days and assessed for evidence of depression and anxiety-likebehaviors during acute abstinence. Compared to saline-treated rats, oxycodone rats showed decreasedsaccharin preference, increased anxiety-like behavior on the elevated plus maze, and reduced mobilityduring the forced swim test (independent samples t-tests, $p<0.001$). Other withdrawal signs such ashyperalgesia and reduced weight gain were also observed (independent samples t-tests, $p<0.05$). Ratswere sacrificed 24h following their final saline or oxycodone treatment, and orexin expression wasassessed using immunohistochemistry. Oxycodone-treated rats (n=7) expressed greater numbers oforexin-expressing neurons compared to saline controls (n=7; independent samples t-test, $p<0.01$).Moreover, oxycodone-treated rats with more orexin neurons exhibited stronger negative affectivebehaviors during acute abstinence compared to saline-treated subjects (*Pearson's r, n=7, r=-0.90)*. Todetermine the functional significance of the orexin system in the development of negative affectivesymptoms, the inhibitory DREADD hM4Di was selectively expressed in orexin neurons of male Bac-transgenic orexin-cre rats. Oxycodone-treated rats expressing hM4Di (n=12) showed attenuated affectivesymptoms during acute abstinence compared to mCherry control rats (n=12; independent samples t-test,$p<0.05$), indicating that the orexin system must be engaged for these behaviors to develop. In sum, thedata support a novel role for the orexin system in affective behaviors associated with opioid withdrawaland further highlight this system as a potential treatment target for OUD.

Disclosures: J.E. Fragale: None. **V.A. Stiritz:** None. **K.A. Newman:** None. **M.H. James:** None. **G.S. Aston-Jones:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.03

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute On Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number T32AA028254

Title: Role of orexin-1 receptor signaling in demand for the opioid fentanyl in adult females

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Abstract: Over 10 million individuals in the United States misuse opioids, and opioids are involved in 70% of drug overdose deaths, which was exacerbated during the COVID-19 pandemic. The opioid epidemic appears to affect women differently than men, indicated by the notable increase of prescription opioid-related deaths among women over the past two decades. The orexin / hypocretin system plays a critical role in reward-processing and is a potential treatment target for drug addiction. Preclinical studies suggest that higher orexin system activity in females and female rodents exhibit higher motivation to self-administer opioids. Fluctuating hormone levels during the menstrual cycle may greatly affect drug seeking behavior, as indicated by morphine's analgesic effect being greater during the follicular phase than the luteal phase of the menstrual cycle. We previously reported that, in male rats, orexin-1 receptor (OxR1) antagonist SB-334867 (SB) decreased demand for fentanyl, a short-acting drug with 50 times the potency of heroin. A behavioral economics (BE) procedure measures drug motivation (inverse α) and drug consumption at null cost (Qo) and has been proven to be useful in the study of opioid abuse, as SB efficacy and the amount of cued reinstatement of fentanyl seeking were predicted by baseline α values in male rats. To gain insight into the role of the orexin-system in fentanyl addiction in females, we assessed the effects of OxR1 antagonism using the BE procedure in adult female Long-Evans rats while monitoring their estrous cycle. In addition, we applied different access paradigms for fentanyl self-administration (1-hr continuous access (short access [ShA]), 6-hr continuous access (long access [LgA]), or 5-min access followed by 25-min of no access for 6-hrs (intermittent access [IntA])) for 14 days, resulting in varying degrees of fentanyl demand. SB efficacy was tested using the BE procedure and its effect on cue-induced fentanyl seeking in female rats. SB significantly decreased motivation (increased α) for fentanyl at the dose of 30 mg/kg B.W. (rm-ANOVA with Dunnett's test; *$p < 0.05$; n=11) without affecting Qo (rm-ANOVA; ns). However, unlike in male rats, SB appears less effective at reducing the fentanyl seeking in highly motivated (Pearson's r; *$p < 0.05$) female rats. Lower α predicts higher cued reinstatement of fentanyl seeking (Pearson's r; $p = 0.10$; n=8) which tends to decrease with SB treatment (rm-ANOVA with Dunnett's test; $p = 0.11$; n=8). Further analysis will be performed to elucidate the role of estrous cycle in fentanyl seeking female rats. In conclusion, our study supports OxR1 antagonist as a potential pharmacotherapy for opioid addiction in women.

Disclosures: C. Corwin: None. **D. De Sa Nogueira:** None. **V. Punnuru:** None. **Y. Rakholia:** None. **J.E. Fragale:** None. **A.S. Kohtz:** None. **G.S. Aston-Jones:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.04

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA006214

Title: Elevated depressive- and anxiety-like behaviors with prolonged abstinence from intermittent fentanyl self-administration

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Abstract: The urgency of the opioid crisis is heightened by a rise in abuse of synthetic opioids. Identifying pharmacological targets for effective opioid addiction treatments, which are scarce, requires animal models recapitulating the multifaceted addicted state. Opioid use disorder (OUD) is marked by a negative emotional state in abstinence (including depressive- and anxiety-like behaviors), the alleviation of which may motivate prolonged drug-seeking. The goal of this study was to assess depressive- and anxiety-like behaviors arising in protracted abstinence from intermittent access (IntA) fentanyl self-administration (SA), which induces strong drug motivation and relapse behaviors. We sought to correlate increased motivation for fentanyl with negative affective and relapse behaviors. Adult male Sprague Dawley rats (n=15) self-administered fentanyl (0.5g iv/infusion) in 2h sessions for 10d and until 3 consecutive sessions resulted in at least 25 infusions. Baseline fentanyl motivation was measured using a within-session behavioral economics (BE) procedure, which assesses drug consumption with increasing price, followed by 14d of fentanyl IntA SA. Post-access fentanyl demand was tested with BE. Rats were tested either in acute withdrawal or following 30d of abstinence for depressive- and anxiety-like behaviors with saccharin preference, social interaction (SI), and light-dark box (LDB) tests and the elevated plus maze (EPM). All rats were re-exposed to the SA chamber for 2h without infusions and cues to test relapse behavior. The abstinence group was tested on BE for persistent changes in fentanyl motivation. Rats showed escalated fentanyl intake during IntA and higher drug motivation with BE post-IntA compared to pre-IntA, indicating induction of a robust addiction-like state. Following 30d of abstinence, rats (n=8) showed decreased saccharin preference and SI scores and increased depressive- and anxiety-like behaviors in the LDB and EPM, respectively, compared to acute withdrawal rats (n=7; independent samples t-tests, $p<0.05$). Early data show that these behaviors are correlated to post-access fentanyl motivation, which was elevated at 30d of abstinence, and that fentanyl motivation predicts relapse behavior, such that the highest-motivated rats show greater relapse when re-exposed to the SA chamber.

These results confirm that IntA induces an expansive addiction-like state including depressive- and anxiety-like behaviors, pointing to its value as a preclinical model of OUD; that a negative emotional state arises with abstinence from opioids; and that drug motivation assessed with BE can predict this state and relapse propensity.

Disclosures: K.A. Newman: None. **J.E. Fragale:** None. **G.S. Aston-Jones:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.05

Topic: G.09. Drugs of Abuse and Addiction

Support: 1K99DA045758
4T32ES007148
R01DA006214
U54DA016511

Title: Oxytocin attenuates progesterone-dependent perturbations in cocaine demand

Authors: *A. KOHTZ, H. DAVIES, B. LIN, J. ZHAO, M. PRESKER, G. ASTON-JONES; Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: There are substantial sex differences in drug abuse, and a key feature of cocaine addiction is pathologically high economic demand for drug. The hypothalamic neuropeptide oxytocin (OXT) is heavily implicated in the modern treatment of substance abuse disorders. Notably, OXT neuron activity is reduced during abstinence from chronic cocaine exposure in both males and females. Female rats may be particularly sensitive to OXT dysfunction following cocaine use, as there are substantial differences in OXT mRNA, binding affinity, and OXT target neuron activity across the estrous cycle that are progesterone (P4)-dependent. Using a within-session threshold behavioral economics (BE) procedure, we quantified demand elasticity (α, inverse motivation) and free consumption (Q_0, hedonic setpoint) in female rats across the estrous cycle and in ovariectomized (OVX) and hormone-replaced female rats. Overall, female rats showed lower demand elasticity (greater motivation) for cocaine compared to males, with estrus cycle phase accounting for 70% of the variance between data points within a subject. High serum P4 (e.g., in proestrus) predicted decreased cocaine motivation, and administering P4 (4 mg/kg) to low P4 females (OVX, diestrus) decreased cocaine motivation. Notably also, long-term cocaine self-administration altered estrous cyclicity; indicating substantial endocrine disruption. By 13 weeks, proestrus epochs were no longer observed, and cocaine demand was potentiated by permanent estrus. Using naturally cycling rats, we found that OXT efficacy to decrease motivation for cocaine is greater during the high-demand phase of the estrous cycle (diestrus) as

compared to low demand phases (proestrus) or male rats that have lower demand overall. Following each individual injection, OXT at either 0.1mg/kg or 0.3mg/kg restored estrous cycling in intact females with prior cocaine experience for a period of one week and was continuously effective with up to 4 weeks of injections. Centrally, activity of OXT neurons in the paraventricular hypothalamic nucleus correlated to both decreased demand in females, and to increased circulating levels of P4. Effects of P4-replacement in OVX females were blocked by Atosiban (1 mg/kg; OXT receptor antagonist). Thus, OXT may represent a viable treatment option, not only for acute suppression of drug demand, but also for its long-term effects to reverse the endocrine disrupting effects of cocaine.

Disclosures: A. Kohtz: None. **H. Davies:** None. **B. Lin:** None. **J. Zhao:** None. **M. Presker:** None. **G. Aston-Jones:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA049837
NIDA R01 DA047265
NIH T32 DA007273

Title: A sex-specific role for PKMζ in oxycodone self-administration

Authors: *M. C. KNOUSE[1], T. M. HOUSER[1], E. A. BIRMINGHAM[1], L. A. BRIAND[2]; [2]Psychology, [1]Temple Univ., Philadelphia, PA

Abstract: The Protein Kinase C (PKC) family works to control the function of other proteins via phosphorylation. One particular member of this family, PKMζ, is constitutively active and found exclusively in the central nervous system. Previous work from our lab demonstrated that constitutive deletion of PKMζ potentiates cocaine self-administration and cue-induced reinstatement in both male and female mice. Additionally, other groups have demonstrated that disrupting PKMζ leads to increased alcohol drinking. 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. In contrast to what has been seen with cocaine self-administration, we find that PKMζ knockout leads to an increase in oxycodone self-administration in female mice at multiple doses tested. However, male PKMζ knockout mice do not exhibit alterations in oxycodone self-administration compared to wildtype mice. We next went on to examine the influence of oxycodone self-administration on synaptic plasticity within the nucleus accumbens of wildtype and PKMζ knockout mice. Preliminary

electrophysiological data indicate that both wildtype and PKMζ knockout mice exhibit normal long-term depression following oxycodone self-administration. Current work is extending these findings to examine presynaptic plasticity with paired-pulse ratios and spontaneous glutatmate transmission with sEPSCs in wildtype and PKMζ knockout mice following oxycodone self-administration. These results suggest that PKMζ plays a sex-specific role in opioid drug taking.

Disclosures: M.C. Knouse: None. **T.M. Houser:** None. **E.A. Birmingham:** None. **L.A. Briand:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA RO1 DA049837
NIDA RO1 DA047265

Title: The role of PKMζ in anxiety-like behavior in male and female mice.

Authors: *A. M. MCBRIDE[1], R. DEARRING[2], R. E. PEREZ TREMBLE[2], E. A. BIRMINGHAM[2], L. A. BRIAND[2];
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Abstract: The constitutively active, atypical protein kinase C, protein kinase M-ζ, is exclusively expressed in the brain and plays a role in drug seeking. Constitutive deletion of PKMζ leads an increase in drug seeking. However, the role of PKMζ in drug seeking is sex-specific, with accumbal PKMζ knockdown only affecting cocaine seeking in male mice, while constitutive deletion increases cocaine seeking in both sexes. Mood disorders are often comorbid with substance use disorder and play a role in both vulnerability to drug use and propensity for drug relapse. Along with its established role in drug seeking, previous research has determined that PKMζ plays a role in anxiety-like behavior as well. However, despite the fact that mood disorders are more prevalent in women, the previous work was conducted only in male rodents, therefore, it is not clear whether the role of PKMζ in anxiety-like behavior is also influenced by biological sex. The goal of the current studies was to determine the role of PKMζ on anxiety-like behavior in male and female mice. We found that constitutive PKMζ deletion led to alterations in the novelty-suppressed feeding task that were sex specific. Studies are underway to determine if these sex-specific findings extend to other tests of anxiety-like behavior. Further, as we have previously found PKMζ deletion within the accumbens plays a role in cocaine seeking in males, we are extending these findings to determine the influence of site-specific manipulations of

PKMζ expression. These findings suggest that PKMζ within the mesolimbic pathway plays a role in anxiety and drug seeking.

Disclosures: A.M. McBride: None. **R. Dearring:** None. **R.E. Perez Tremble:** None. **E.A. Birmingham:** None. **L.A. Briand:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA047265
NIDA Grant R01 DA049837

Title: Post-weaning social isolation alters addiction-like behaviors and synaptic plasticity in the nucleus accumbens and prefrontal cortex: Role of sex and neuroimmune signaling.

Authors: *A. G. MCGRATH, B. T. RHOADS, E. A. BIRMINGHAM, M. C. KNOUSE, L. A. BRIAND;
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Abstract: Mitigation efforts during the coronavirus pandemic led to an increase in social isolation. This can be particularly difficult for adolescents, when social interaction with peers is particularly rewarding. Animal models have demonstrated that social isolation during adolescence can have long lasting effects. Our lab has shown that post-weaning social isolation leads to increased motivation for cocaine and greater vulnerability to cue-induced relapse in adulthood. However, little is known about how post-weaning social isolation alters the brain. Early life stress can lead to persistent restructuring of neurons. Changes in dendritic spines can be long-lasting and have been theorized to play an important role in the maintenance of cocaine craving even after long periods of abstinence. In the current study, we found that post-weaning social isolation stress led to a persistent increase in spine density in adulthood within both the core and shell regions of the nucleus accumbens in male mice, but not female mice. In contrast, post-weaning isolation increased spine density within the prelimbic cortex of both males and females. Further, in the infralimbic cortex, post-weaning social isolation led to an increase in spine density only in female mice. This study highlights the long-lasting, sex-specific effects of post-weaning isolation, however the mechanisms by which isolation alters spine density remains unknown. Microglia have been shown to assist in both the formation and elimination of dendritic spines, and are activated following exposure to stress and cocaine. Therefore, we hypothesized that microglia may be involved in the restructuring of dendritic spines during post-weaning isolation, and contribute to addiction-like behavior in adulthood. We examined whether

inhibiting microglia with minocycline during the first three weeks of post-weaning isolation would alter the impact of isolation in cocaine seeking. Isolated animals that received minocycline showed increased cocaine seeking in adulthood compared to group housed mice and isolated mice that received saline. This increased cocaine seeking was associated with increased spine density in the nucleus accumbens. The findings of these studies provide insight into the mechanisms by which social isolation during adolescence increases vulnerability to addiction later in life.

Disclosures: A.G. McGrath: None. **B.T. Rhoads:** None. **E.A. Birmingham:** None. **M.C. Knouse:** None. **L.A. Briand:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA049837
NIDA R01 DA047265

Title: Post-weaning social isolation alters sociability in male mice but not female mice.

Authors: *E. A. BIRMINGHAM[1], B. T. RHOADS[1], A. G. MCGRATH[1], N. A. MILES[1], L. A. BRIAND[2];
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Abstract: Adolescence is a critical period for brain development and social adversity during adolescence can have long lasting impacts on mental health. Social reward is particularly salient during adolescence and a lack of social interaction can lead to long-lasting effects on behavior and brain development. Social isolation and loneliness may lead to more detrimental mental health outcomes in women[14] however, much of the work on the impact of social isolation has been performed exclusively in male animals. Our lab has previously shown that post-weaning social isolation increases vulnerability to reinstatement of cocaine seeking in adulthood and this impact of social isolation does not extend to reinstatement of natural rewards. However, to date, it is not known whether this social isolation paradigm influences social reward behavior. The goal of these experiments was to examine the effects of post-weaning social isolation on sociability and social reward both during adolescence and adulthood. First, we examined the impact of housing condition on a three-chamber sociability task during adolescence at PND45 (24 days after the start of social isolation). We found that adolescent male mice spend more time sniffing a novel mouse than a novel object and this preference for social interaction was not influenced by their housing condition. In contrast adolescent female mice spend equal times

interacting with the novel mouse and the novel object. Overall, female mice that were socially isolated and left unhandled in their cage exhibited lower novelty seeking than group housed females. When the mice were tested during adulthood, we found that male mice that were socially isolated at weaning maintained this preference for interacting with a novel social partner, whereas male group housed animals did not. Current work is underway to extend these findings to examine social reward and social recognition memory in male and female mice following post-weaning social isolation.

Disclosures: E.A. Birmingham: None. **B.T. Rhoads:** None. **A.G. McGrath:** None. **N.A. Miles:** None. **L.A. Briand:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA R36 DA05095
NIH/NIDA R01 DA039146
NIDA- and NIAAA-IRPs

Title: Impact of access conditions on substance use disorder-like phenotypes and receptor densities in rats self-administering cocaine or 3,4-methylenedioxypyrovalerone

Authors: *M. R. DOYLE[1,3], G. G. GOULD[2], M. S. BUSHNELL[1], M. SYED[1], K. C. RICE[4], L. C. DAWS[2,1], G. T. COLLINS[1,3];
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Abstract: Despite decades of research, the behavioral, pharmacological, and neurobiological determinants of one's vulnerability to develop a substance use disorder (SUD) are not well understood. When rats self-administer cocaine under short-access conditions, patterns of drug-taking tend to be well-regulated; however, manipulating drug access conditions (e.g., long-access or intermittent-access) can produce neurobiological and behavioral changes thought to be related to SUD. In contrast to cocaine, when rats self-administer the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV) under short-access conditions, a subset of rats rapidly develop high levels of dysregulated drug-taking behavior. MDPV has not been studied under extended access conditions, and it is unknown whether this MDPV phenotype is an earlier manifestation of the SUD-like phenotype observed with extended access to cocaine, or whether the two phenotypes are qualitatively different. Additionally, changes in receptor densities (e.g.,

dopamine D_2, dopamine D_3, 5-HT_{2A} or 5-HT_{2C}) in rats with more severe SUD-like phenotypes are not well characterized. The current studies used male and female Sprague Dawley rats self-administering MDPV (0.032 mg/kg/infusion) or cocaine (0.32 mg/kg/infusion) to test the hypotheses that (1) under short-access conditions, rats self-administering MDPV exhibit higher levels of drug taking, greater levels of responding when drug is not available, and reduced sensitivity to punishment by foot shock (i.e., SUD-like behaviors); (2) that 21 days of long- and intermittent-access to MDPV or cocaine self-administration will result in greater SUD-like behaviors compared to short-access self-administration; and (3) differences in receptor densities will be related to the SUD-like phenotype score. After short-access self-administration, rats that self-administer MDPV exhibited greater SUD-related behaviors than rats self-administering cocaine. Though long- and intermittent-access altered patterns and/or levels of drug-taking behavior, manipulating access condition did not appear to systematically alter the SUD-like behaviors or receptor densities in rats self-administering either cocaine or MDPV. Early analyses suggest no difference in receptor densities among groups, however quantification is in early stages. These results suggest that the phenotype exhibited by rats self-administering MDPV is not the same phenotype as that observed for rats self-administering cocaine. Therefore, short-access MDPV self-administration may be a useful method to study individual differences in drug-taking behavior that may be related to SUD in humans.

Disclosures: M.R. Doyle: None. **G.G. Gould:** None. **M.S. Bushnell:** None. **M. Syed:** None. **K.C. Rice:** None. **L.C. Daws:** None. **G.T. Collins:** None.

Poster

P727. Addictive Drugs: Neural Mechanisms

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P727.11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS-NIH P30GM122733

Title: Intermittent social defeat stress decreases the neuronal response to reward-predicting cues in the prefrontal cortex during a reward-seeking task

Authors: H. M. HARRIS[1], ***A. DEL ARCO**[2];
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Abstract: The intermittent (episodic) exposure to social defeat stress (ISD) increases cocaine self-administration and makes animals more vulnerable to develop substance use disorders. Previous studies suggest that an increased reward-seeking behavior is associated with an impaired reward processing in the prefrontal cortex (PFC). The present study tests the hypothesis that ISD impairs reward processing in the PFC by recording cue-evoked neuronal activity in this

area of the brain. Male Long Evans rats (3-4 months old) were trained in a discriminative-stimulus (DS) reward-seeking task in which rats were required to engage in or withhold responding on trial-by-trial basis depending on a specific stimulus. Briefly, rats were trained to discriminate between a rewarded (continuous tone) and a non-rewarded (intermittent tone) stimulus that was paired to the extension of a lever (right or left, counterbalanced). Responding to the rewarded cue by pressing the lever delivered a sugar pellet. After stable performance, rats were implanted with multiunit electrode arrays in the medial PFC (prelimbic area) and divided in two groups (Control, n= 7; Stress, n= 8). Then, they were exposed to ISD (or handling, Control group) once every three days for ten days (four stress episodes in total). PFC activity was recorded during the DS task in both stressed and control animals two weeks after the last stress episode. The results show that both stressed and control animals made more responses (lever presses) to the rewarded cue compared to the non-rewarded cue across sessions ($F_{(7,13)}$= 34.37; $p< 0.001$, ANOVA). There were not group differences in the number of lever presses in response to both cues. The onset of the reward cue produced the activation and inhibition of different neuronal populations in the PFC. Critically, the number of activated neurons in response to the reward cue was significantly lower in stressed animals compared to controls ($\chi^2(2)= 10.64$, $p< 0.01$). Moreover, and in contrast to controls, stressed animals showed an inhibition of the average population activity in response to the cue. These results show that ISD alters cue-evoked prefrontal neuronal activity in the long term (weeks after stress) and suggest that a decreased sensitivity to reward-predicting cues in the PFC increases vulnerability to develop drug abuse.
Supported by NIGMS-NIH P30GM122733.

Disclosures: H.M. Harris: None. **A. Del Arco:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA043190
NIH AA007583

Title: Activation of gaba projection neurons from the ventral tegmental area to the nucleus accumbens facilitates cocaine self-administration at low doses

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Abstract: Several brain areas play a role in sensitivity to both cocaine and cocaine-associated cues; the Ventral Tegmental Area (VTA) is heterogeneous midbrain region with dense afferents

to the Nucleus Accumbens (NAc) that has been extensively studied for its role in natural- and cocaine-associated behaviors. Most of the neurons in the VTA are dopaminergic, however, the VTA also contains GABA interneurons, as well as GABA projection neurons (GPNs). Approximately one third of mesoaccumbal afferents are GABAergic and are critical for cue-motivated responding. Using a combinatorial viral approach to target activating designer receptors exclusively activated by designer drugs (DREADDs) to VTA glutamate decarboxylase 1-positive neurons in rats, our lab has previously demonstrated that chemogenetic global activation of all VTA GABA neurons decreases motivation for sucrose-predictive cues. Furthermore, when reward value was unexpectedly reduced by decreasing the volume of sucrose earned, clozapine N-oxide (CNO) microinfused only in the NAc reduced responding to the sucrose-predictive cue when compared to controls. Currently, the role of these GPNs in cocaine-mediated behaviors is still largely unknown. Rats were trained to self-administer cocaine (0.5 mg/kg/inf), by lever pressing, for 10 days. A dose-response curve was then generated by allowing rats to self-administer one of five doses (0.09, 0.18, 0.37, 0.75, or 1.5 mg/kg/inf) every other day (maintaining on training dose in between). We chemogenetically activated either all VTA GABA neurons by giving the CNO systemically (0.3 mg/kg i.p.), or just the VTA GABA neuron terminals in the NAc by microinfusion of CNO. We then trained a separate group of rats to train for 5 days (0.5 mg/kg/inf) to establish responding, then changed them to a progressive ratio to evaluate break-points at the same five doses for the dose response, splitting the rats into the same treatment conditions as before. Our results show that a descending dose-response curve is readily generated, with rats lever pressing the most at the lowest dose, and the least at the highest dose. Systemic CNO decreased responding on the active lever only the two lowest doses. Activating only accumbal terminals of VTA GABA projection neurons, increased responding on the two lowest doses. A similar result was seen at the lowest dose of break points on progressive ratio. CNO had no effect in virus control rats. These results clearly establish that mesoaccumbal GABA neurotransmission facilitates either a reduction in motivation to seek and take drug, after global VTA GABA activation, or an increase following local terminal activation at the level of the nucleus accumbens.

Disclosures: M. Suarez: None. **C.E. Bass:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01DA041455
NIH T32DA007244

Title: Effects of chemogenetic nucleus accumbens astrocyte stimulation on the incubation of cocaine craving

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Abstract: Astrocytes play important and complex roles in the modulation of behavior, including those involved in neuropsychiatric disease. Previous data from our lab has found that astrocytes in the nucleus accumbens (NAc) core undergo changes in surface area and synaptic colocalization following cocaine self-administration and extinction (Testen et al., 2018, Scofield et al., 2016). Further unpublished data has indicated similar, but more pronounced structural changes following incubation of cocaine craving, a clinically-relevant phenomenon characterized by an increase in behavioral measures of drug seeking across abstinence (Kim et al., SfN 2019), raising the hypothesis that normalization of astrocyte structure and activity may oppose cocaine-induced adaptations. Chemogenetic stimulation of astrocytes has been explored as a means of reducing cocaine seeking after self-administration due to its potential for stimulating transmitter release from astrocytes and its potential for increasing their complexity (Chen et al., 2016) and cell body area (Brockett et al., 2018). While chemogenetic stimulation of NAc core astrocytes was found to be successful at preventing cue-induced cocaine reinstatement (Scofield et al., 2015), the same has not been examined in the context of an incubation of craving model. To address this question, we first tested astrocyte-specific expression of two commercially available DREADDs in the rat nucleus accumbens: AAV5-GFAP-hM3Dq-mCherry (Addgene cat #50478) and AAV5-GfaABC1D-hM3Dq-mCherry (Addgene cat #92284). We observed significantly greater specificity and efficiency of transduction in astrocytes with AAV5-GfaABC1D-hM3Dq-mCherry (Addgene cat #92284, astrocyte specificity: 92.1-99.3%) relative to AAV5-GFAP-hM3Dq-mCherry (Addgene cat #50478, astrocyte specificity: 34.5-80.6%). We observed that that diluting AAV5-GFAP-hM3Dq-mCherry (Addgene cat #50478) resulted in greater specificity and efficiency of transduction in astrocytes. We next tested effects of these AAVs versus AAV5-GFAP-mCherry following 10 days of long-access (6h/day) cocaine self-administration followed by 45 days of home-cage abstinence. Prior to a cue-primed seeking extinction test, animals received either CNO or vehicle treatment. Results indicated that animals that received AAV5-GFAP-hM3Dq-mCherry (cat #50478) showed significantly reduced cocaine seeking relative to all other control groups. Ongoing experiments are evaluating the effects of AAV5-GfaABC1D-hM3Dq-mCherry.

Disclosures: E.V. Harder: None. **A. Testen:** None. **K.J. Reissner:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA049139 (RTL)
NIH Grant DA048055 (RTL)

Title: Rat infralimbic projections to the nucleus accumbens shell encode cocaine extinction contingencies during temporally specific windows

Authors: *K. E. NETT[1,2], M. S. MCGREGOR[1,2], A. R. ZIMBELMAN[1], R. T. LALUMIERE[1,2,3];
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Abstract: Prior evidence indicates that the medial prefrontal cortex regulates cocaine-seeking behavior in rats. In particular, studies suggest that the ventral portion of this region, known as the infralimbic cortex (IL), mediates the ongoing inhibition of cocaine seeking, specifically through its projections to the nucleus accumbens (NA) shell. Our own data indicates IL activity during the 20 s immediately following an unreinforced lever press is critical for extinction encoding. However, whether signaling from the IL to the NA shell encodes extinction learning remains unclear. Moreover, it is unknown whether the critical period for this encoding is limited to the initial 20 s period after a lever press or extends longer. To address this issue, we used an activity-controlled, “closed-loop” optogenetic approach in which IL-NAshell activity was silenced following an unreinforced lever press for the first 20 s (0-20 s) or for the second 20 s after the lever press (20-40 s) during extinction training. Male and female Sprague-Dawley rats received bilateral injections of AAV-CaMKII-eArchT3.0 into the IL and bilateral fiber optic probes above the NAshell. Rats then underwent two weeks of daily 2 h cocaine self-administration sessions, in which active lever presses produced a cocaine infusion (0.33 mg/kg/inf), light/tone cues, and retraction of the active lever for 20 s or 40 s. Following this, rats underwent 5 d of manipulated extinction training (30 min sessions), in which active lever presses yielded no cocaine or cues but led to lever retraction (20 s or 40 s) and bilateral illumination of IL terminals in the NAshell (from 0-20 s or 20-40 s). Following those 5 days with inhibition, each rat underwent 7 d of full length (2 h), unmanipulated extinction sessions in which lever presses only resulted in a lever retraction for 20 or 40 s. The results indicate that IL-NAshell inhibition from 0-20 s post lever press, and not 20-40 s post lever press, significantly impairs extinction learning during the manipulated sessions and the retention of this extinction learning, as assessed during the unmanipulated sessions. These findings point to a critical window following an unreinforced lever press during which activity from IL inputs to the NAshell encodes the new extinction contingencies between the lever press and lack of a cocaine reinforcer. Moreover, combined with previous work, the results suggest that plasticity underlying extinction encoding occurs, at least in part, beyond the IL itself and in the synaptic inputs to the NAshell.

Disclosures: K.E. Nett: None. **M.S. McGregor:** None. **A.R. Zimbelman:** None. **R.T. LaLumiere:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant DA046457

Title: Investigating the role of the intralaminar nuclei of the thalamus in compulsive cocaine seeking in rats

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Abstract: Compulsive drug seeking that continues despite negative consequences may result from a loss of behavioral flexibility. The intralaminar nuclei of the thalamus (ILN), which is comprised of parafascicular, centromedian, centrolateral, and paracentral nuclei, has been implicated in behavioral flexibility. Therefore, we hypothesized that ILN may play a role in maintaining flexible control over drug seeking when faced with negative consequences. To determine the role of ILN in compulsive cocaine seeking, we tested the effects of post-training lesions of ILN on sensitivity to footshock punishment. Male Sprague Dawley rats were trained to self-administer cocaine on a seeking-taking chained schedule of reinforcement during daily 2-hour sessions for ~3 weeks. The rats then received NMDA or sham lesions of bilateral ILN and resumed self-administration following recovery from surgery. To assess compulsive cocaine seeking, rats were first given 4 days of mild footshock (0.4 mA, 0.3 s, randomly 1/3 of trials) upon completion of the seeking link of the chain during self-administration. Following recovery from punishment, rats were then given a second round of ramped footshock in which there was a quarter-log increase in footshock amplitude each day (0.18 mA, 0.32 mA, 0.56 mA, 1.0 mA, 0.3 s, every trial) to determine a threshold for sensitivity. With both types of footshock punishment testing, we observed no difference in punishment sensitivity in rats that received ILN lesions, as compared to rats with sham lesions. These results indicate that, despite a role in behavioral flexibility, ILN is not involved in flexibility related to footshock-induced suppression of cocaine seeking.

Disclosures: A.M. Cruz: None. **R.J. Smith:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505 (JW)
NIAAA U01AA025932 (JW)

Title: Drug Reinforcement Impairs Cognitive Flexibility via Inhibiting Striatal Cholinergic Neurons

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Abstract: Reinforcing behaviors are associated with deficits in cognitive flexibility, wherein a subject is required to extinguish an old behavioral strategy and explore a new one if the old strategy is no longer rewarding. The neural circuitry underlying this flexibility deficit is not known. Cholinergic interneurons (CINs) in the dorsomedial striatum (DMS) are known to play a critical role in behavioral flexibility. In this study, we used chronic cocaine and alcohol exposure as reinforcement models to investigate the role of DMS CINs in the reversal of instrumental learning. We found that chronic cocaine or alcohol exposure increased GABAergic inputs to DMS CINs and reduced their spontaneous firing activity. Morphological and physiological studies demonstrate that the major GABAergic input to the DMS CIN originates from the direct-pathway medium spiny neurons (dMSNs) and this input was potentiated by chronic cocaine or alcohol exposure. To artificially mimic the inhibition of DMS CINs, we employed chemogenetic and time-locked optogenetic approaches to downregulate DMS CIN activity, which led to deficits in behavioral flexibility. The dMSN to SNr (Substantia Nigra Reticulata) pathway is known to mediate reinforcing behaviors. Functionally, we test that dMSNs also send collateral inhibitory connections to the CINs. Therefore, we propose that, on one hand dMSN to SNr pathway causes reinforcement, at the same time dMSN to CIN pathway leads to inflexibility, thus providing the neural substrate for deficits in flexibility caused by reinforcing behaviors.

Disclosures: H. Gangal: None. **Y. Cheng:** None. **X. Xie:** None. **X. Wang:** None. **J. Lu:** None. **X. Zhuang:** None. **Y. Huang:** None. **W. Wang:** None. **L.N. Smith:** None. **J. Wang:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.06

Topic: G.09. Drugs of Abuse and Addiction

Support: DA045308

Title: The Role of the Patch Compartment of Striatum in Reward-Driven Behaviors.

Authors: *J. AHN[1], D. J. CHRISTY[2], K. HORNER[2];
[1]Master of Biomed. Sci. Program, [2]Biomed. Sci., Mercer Univ. Sch. of Med., Macon, GA

Abstract: The striatum is a neural structure that plays a critical role in cognitive functions, behavioral decision-making, and reward generation. The striatum exhibits a heterogeneous composition, containing neurons belonging to the patch compartment—which is thought to be involved in habitual reward-related behaviors—surrounded by neurons belonging to the matrix compartment—which is thought to be involved in adaptive motor control. Additionally, the striatum is further subdivided into the dorsolateral striatum (DLS) and the dorsomedial striatum (DMS), each with their own patch and matrix compartments. The DMS has been associated with goal-oriented behavior seen during the initial stages of addiction. Conversely, the DLS has been associated with habitual behaviors seen during late-stage addictive behaviors that are inflexible. It is thought that drug addiction is initially mediated by the DMS before DLS activity becomes predominant. Previously, it has been shown that the patch compartment of the DLS is necessary for development of habitual behavior, but the role of the patch compartment of the DMS is less clear. Our study intends to demonstrate that selective ablation of DMS patch compartment neurons will result in a negative impact on the initial development of reward-driven behaviors during the early stages of drug addiction. Since patch compartment neurons express a high level of mu opioid receptors compared to the surrounding matrix, we used dermorphin-saporin, a toxin that selectively destroys mu opioid receptor-containing neurons to target patch compartment neurons in the DMS and DLS for ablation. Following infusion in the DMS or DLS with dermorphin-saporin (17 ng/μl) or vehicle, rats were trained to self-administer cocaine (0.4 mg/kg/infusion) on progressive ratio schedule of reinforcement, starting with fixed ratio of 1 and ending with a fixed ratio of 5. Ablation of the patch compartment altered the level of responding for cocaine as the schedule of reinforcement became progressively labor-intensive. These data suggest that the patch compartment contributes to reward-driven behaviors.

Disclosures: J. Ahn: None. **D.J. Christy:** None. **K. Horner:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Activation of the basolateral amygdala alters rearing-induced changes in cocaine-seeking

Authors: A. BROWN[1], K. FROIDCOEUR[1], C. SANTAMARIA[2], M. WOHRLEY[1], J. GREENBERG[1], M. VOGEL[1], M. LINDEN[1], R. HEBRON[1], ***M. GILL**[1];
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Abstract: Early environmental rearing impacts susceptibility to drug abuse later in life. Rearing rats in enriched (EC), impoverished (IC), or social (SC) conditions results in neurobiological, neurochemical, and behavioral changes (Bowling, Rowlett, & Bardo, 1993). In EC rats, these rearing-induced changes result in a protective effect, as EC rats self-administer less drug at low doses and reinstate drug seeking at lower rates than IC and SC rats. We hypothesize that the glutamatergic system is responsible for this protective effect based on previous work implicating accumbal GluA1 in cocaine self-administration (Pierce & Wolf, 2013) and reinstatement (Anderson et al., 2008). Previous work from our lab implicates the nucleus accumbens shell (NAshell) in reversing rearing-induced reinstatement differences, but the glutamatergic input from the infralimbic cortex (IL) only partially accounts for these differences; suggesting a secondary input is impacting the NAshell. The basolateral amygdala (BLA) receives glutamatergic input from the IL, and projects to the NAshell, and previous work shows that inactivation of the BLA attenuates cue-induced reinstatement (McLauglin & See, 2003). Additionally, environmental enrichment reverses cocaine incubation-induced molecular changes in the BLA-NA pathway when used as an intervention (Ma et al., 2016). Based on these findings, the current study focuses on the impact of the BLA on cocaine seeking in differentially reared rats. Specifically, PEPA, an AMPA positive allosteric modulator, was infused into the BLA immediately prior to reinstatement of cocaine seeking behavior. Male Sprague-Dawley rats arrived at 21 days of age, and were reared in EC, IC, or SC contexts for 30 days. Beginning at 52 days of age, were trained using a standard 2-hr cocaine self-administration model including extinction, and reinstatement. Rats were pseudo-randomly assigned to receive PBS or PEPA infusions into the BLA prior to cocaine-primed, cue-induced or yohimbine plus cue-induced reinstatement tests. Results show an attenuation of rearing-induced reinstatement differences following cue-induced and yohimbine plus cue reinstatement tests.

Disclosures: A. Brown: None. **K. Froidcoeur:** None. **C. Santamaria:** None. **M. Wohrley:** None. **J. Greenberg:** None. **M. Vogel:** None. **M. Linden:** None. **R. Hebron:** None. **M. Gill:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA051662
NIH Grant DA042029

Title: Investigating neural activity in the rodent lateral amygdala during and after cocaine-self administration

Authors: *J. KLEINBART[1], S. J. STRINGFIELD[2], A. K. FELDMAN[3], M. M. TORREGROSSA[2], D. J. WEBER[4];
[1]Dept. of Bioengineering, [2]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [4]Mechanical Engin., [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: While viable pharmacological interventions exist for certain substance use disorders (SUDs), there is currently no reliable intervention for psychostimulant use disorders. SUDs are known to affect learning and memory processes, but little is known about neural mechanisms of drug memory formation. By seeking a better understanding of the mechanisms through which neutral sensory cues become associated with psychostimulant use, we aim to identify targets for future interventions for SUDs. Rats were implanted with two-shank 32-contact depth electrodes (NeuroNexus) for recordings in the lateral amygdala (LA). Rats were then trained to self-administer cocaine where active lever presses led to a cocaine infusion and presentation of a 10 second audio-visual stimulus. Following acquisition, rats underwent a cue extinction procedure where the audio-visual cue was presented 120 times in the absence of lever in order to weaken the association between sensory cues and drug reward. Spiking and LFP activity were analyzed in order to look for changes in neural signaling in the LA that occur during memory formation (self-administration) and memory weakening (cue extinction).
Current density analysis of signals recorded on each probe shank during self-administration revealed differential activity along the depth of the probe, suggesting that LA activity is not uniform; this aligns with expectations, as the LA is not organized into layers. Spectrograms depicting RMS power of low-frequency (<300 Hz) signal components on each electrode were produced and selected channels were examined for cue-driven activity by calculating and comparing mean RMS power of early-session lever presses with late-session lever presses. Preliminary data reveal late-session driven responses that appear time-locked to audio-visual stimulus onset and offset. Cue extinction data were similarly analyzed; cue-driven responses present during self-administration appear to dissipate during cue extinction, suggesting a neural mechanism of drug memory encoding and extinction. Additional preliminary spike bandwidth analyses of self-administration trials show consistent spike firing rate increases around 5 seconds after audio-visual cue onset, while cue extinction analysis reveals early session firing rate increases at cue onset and 5 seconds after cue onset and late-session absence of time-locked responses. Taken together, these results suggest that the LA could serve as a viable target for intervention therapies due to the presence of robust but reversible changes in signaling during cue-related drug seeking.

Disclosures: J. Kleinbart: None. **S.J. Stringfield:** None. **A.K. Feldman:** None. **M.M. Torregrossa:** None. **D.J. Weber:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R00DA037271
MSU GE100332

Title: Adolescent cocaine-associated contextual relapse: pattern of neuronal activation in the prefrontal cortex

Authors: D. I. OLEKANMA, B. CHO, A. HERRERA CHARPENTIER, C. REEVES, A. A. ARGUELLO;
Psychology, Michigan State Univ., East Lansing, MI

Abstract: Relapse to drug use is a major problem associated with Substance Use Disorders (SUDs). In 2017, there were approximately 2 million adolescent users of illicit drugs (age 12-17). Initiation of drug use during adolescence can lead to a lifelong risk to develop SUDs, potentially due to strengthened drug-associated stimuli that elicit craving over the lifespan. Previous research using adult rodent models of relapse have shown that in response to drug-associated cues, drug-seeking behavior increases with abstinence time. This incubation effect has also been observed in adolescent rodents: both adolescent and adult rats show a time-dependent increase in seeking behavior. Furthermore, time-dependent, drug seeking behavior in response to drug-associated cues correlated with elevated neuronal activation (cFos) in the medial prefrontal cortex (mPFC) in both adolescent and adult rats.
However, much less is known about the brain regions that are recruited during context-induced cocaine-seeking during adolescence. To determine the pattern of neuronal activation during adolescent relapse, we used an abbreviated rodent model of cocaine self-administration, which allows us to examine relapse-like behavior during the adolescence and adulthood.
Adolescent (postnatal day 32 (P32)) male Sprague Dawley rats received jugular catheterization surgery. Following a recovery period, rats received cocaine self-administration (Coc-SA) training in one context (2h, 2x/d, minimum 10 sessions), followed by extinction (EXT) training in a second distinct context (2h, 2x/d, minimum 8 sessions). Following the last session of EXT, one group of rats received a 2h test in the EXT context. Two additional groups received 2h tests in the cocaine-paired context, 1 day (Test 1) or 15 days (Test 15) after the last EXT session. Adolescent rats displayed an increase in drug-seeking behavior upon re-exposure to the Coc-SA context compared to the EXT context. Furthermore, drug-seeking was significantly higher in rats tested in the cocaine-paired context at Test 15 compared to Test 1. Preliminary neuronal activation data showed a time-dependent increase in cFos expressing cells in the prelimbic cortex correlated with drug-seeking behavior.
Context-driven cocaine relapse occurs during adolescence and is correlated with increased

neuronal activation in the prelimbic region of the PFC. Future directions will examine whether the pattern of relapse-induced neuronal activation differs between adolescent and adult rats across other related brain regions including the orbitofrontal cortex (OFC), basolateral amygdala (BLA) and nucleus accumbens (NAc).

Disclosures: D.I. Olekanma: None. **B. Cho:** None. **A. Herrera Charpentier:** None. **C. Reeves:** None. **A.A. Arguello:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA044204
NIH Grant T32 DA7281

Title: Cocaine-induced nucleus accumbens glutamatergic plasticity in males and females

Authors: *A. M. FRANCE[1], T. L. FETTERLY[1], A. M. NIETO[1], T. E. ROBINSON[2], C. R. FERRARIO[1,2];
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Abstract: The development and persistence of addiction is mediated in part by drug-induced alterations in the function of the nucleus accumbens (NAc), a region that mediates drug-craving and seeking behaviors. AMPA-type glutamate receptors provide the main source of excitatory drive to the NAc, and enhancements in transmission of calcium-permeable AMPAR (CP-AMPARs) within the NAc mediate cue-triggered drug-seeking following long access self-administration. Previous work has shown that cocaine treatment regimens that result in psychomotor sensitization enhance subsequent drug-seeking and drug-taking behaviors. Furthermore, cocaine-induced locomotor sensitization followed by 14 days of withdrawal is accompanied by increases in the surface expression of GluA1 and GluA2 AMPAR subunits (consistent with increases in non-CP-AMPARs) and increases in AMPA/NMDA ratio in males. However, potential effects of cocaine-induced sensitization on glutamatergic transmission in females is poorly understood. In addition, recent studies show that intermittent access self-administration produces robust psychomotor sensitization compared to long access, but effects on NAc glutamate transmission are unknown. We examined, therefore, the effects of experimenter administered cocaine (15 mg/kg, i.p, 8 days) or intermittent vs long-access cocaine self-administration on NAc glutamate. Whole-cell patch clamp approaches were used to measure effects on spontaneous excitatory post synaptic current (sEPSC) frequency and amplitude, and sensitivity to the CP-AMPAR antagonist Naspm (200 μM). Cocaine injections induced

locomotor sensitization in males and females, but enhancements in NAc glutamate transmission were only found only in males. In addition, preliminary results suggest that intermittent access produces enhancements in NAc glutamate transmission that are distinct from those seen following long access. These results will be discussed in light of established sex differences in the induction and expression of psychomotor sensitization, and the ability of intermittent access to produce more robust addiction-like behaviors despite much less total drug consumption.

Disclosures: A.M. France: None. **T.L. Fetterly:** None. **A.M. Nieto:** None. **T.E. Robinson:** None. **C.R. Ferrario:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.11

Topic: G.09. Drugs of Abuse and Addiction

Support: RO1 DA049930
F32 DA046141

Title: Retinoic acid signaling and AMPA receptor plasticity during the incubation of cocaine craving

Authors: *E.-K. HWANG[1], A. M. WUNSCH[1], D. T. CHRISTIAN[2], M. E. WOLF[1];
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA

Abstract: Incubation of cocaine craving refers to the progressive intensification of cue-induced craving that occurs during forced abstinence from cocaine self-administration in rats, and has also been observed in abstinent cocaine users. A key adaptation mediating the expression of incubated craving is accumulation of GluA2-lacking calcium-permeable AMPARs (CP-AMPARs; homomeric GluA1) in excitatory synapses on medium spiny neurons (MSN) in the nucleus accumbens core (NAcc). CP-AMPARs are present at very low levels in NAcc MSN of drug-naïve rats. A rise in their synaptic levels is first detected after several weeks of abstinence; after this time, CP-AMPAR levels remain high for months. Once CP-AMPAR levels are elevated, blocking or removing them from NAcc synapses reduces the expression of incubated craving for cocaine. We are testing the hypothesis that CP-AMPAR upregulation represents a homeostatic response to the contrast between high activity in the reward circuitry during drug self-administration and substantially lower activity during abstinence. Specifically, we hypothesize that a form of retinoic acid (RA)-dependent homeostatic plasticity previously described in hippocampus - in which reduced cellular calcium levels lead to disinhibition of RA synthesis, which in turn disinhibits GluA1 translation and promotes synaptic insertion of

homomeric GluA1 receptors (see L. Chen et al., Neuropharmacology 2014) - is responsible for CP-AMPAR upregulation during incubation of craving. Here we tested the ability of RA to modulate CP-AMPAR levels in MSN of the NAcc. Whole-cell patch clamp recordings were performed in MSN from drug-naïve rats or rats that underwent extended-access cocaine or saline self-administration and >40 days of forced abstinence. In MSN from drug-naïve rats, preincubation of slices for 1 h coupled with bath application of RA (2 μM) resulted in synaptic incorporation of CP-AMPARs, detected as a significant increase in the rectification index (RI) and greater sensitivity to the CP-AMPAR antagonist naspm. This was also observed in response to the retinoic acid receptor α (RARα) selective agonist AM580. The paired pulse ratio was not altered. In cocaine rats, preliminary data indicate occlusion of the RA-induced increase in the RI. Furthermore, preincubation (1 h) of slices with 50 μM DEAB, an inhibitor of RA synthesis, significantly decreased the RI in cocaine rats. These results indicate regulation of synaptic CP-AMPAR levels by RA signaling. Studies are being extended to distinguish D1 and A2a/D2 receptor expressing MSN using transgenic rats expressing Cre recombinase in each MSN population bred to reporter lines.

Disclosures: E. Hwang: None. **A.M. Wunsch:** None. **D.T. Christian:** None. **M.E. Wolf:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA015835
NIH Grant DA049930
NIH Grant DA007262
NIH Grant DA050457

Title: The role of protein translation in D1 and D2-receptor containing neurons of the nucleus accumbens in the expression of incubation of cocaine craving

Authors: *A. B. KAWA[1], E.-K. HWANG[1], A. R. NECTOW[2], H. ZHOU[3], M. COSTA-MATTIOLI[3], M. E. WOLF[1];
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Dept. of Med., Columbia Univ., New York, NY; [3]Baylor Col. of Med., Houston, TX

Abstract: In the ‘incubation of cocaine craving’ model of relapse, rats self-administer cocaine using an extended access procedure, and then experience a prolonged abstinence period. During abstinence, rats exhibit a progressive intensification (incubation) of cue-induced craving. We have shown that Ca^{2+}-permeable AMPA receptors (CP-AMPAR), comprised exclusively of the

GluA1 subunit, accumulate in the nucleus accumbens (NAc) during abstinence and are required for the expression of incubated craving. Further, these CP-AMPARs can be removed from the synapse via mGlu1 positive allosteric modulation, and this treatment reduces incubated seeking. Work in other brain regions has shown that CP-AMPARs can be regulated by the Integrated Stress Response (ISR) pathway. Cellular consequences of activating the ISR pathway include increased phosphorylation of eukaryotic initiation factor 2α (eIF2α). Increased levels of p-eIF2α decrease general mRNA translation but increase translation of a subset of mRNA with 5' upstream open reading frames, such as Oligophrenin-1 (OPHN1). Thus, OPHN1 translation is positively regulated by p-eIF2α. In the hippocampus, OPHN1 is necessary for eIF2α-mediated mGluR-LTD and the removal of synaptic AMPARs. In the VTA, this pathway plays a role in bidirectional CP-AMPAR plasticity in cocaine-exposed animals. Thus, in our NAc studies, mGlu1 stimulation may increase p-eIF2α, leading to increased translation of OPHN1 and the removal of synaptic CP-AMPARs. We are testing this hypothesis by bidirectionally manipulating OPHN1 using acute pharmacological manipulations and chronic viral manipulations. Studies to date show that knocking down OPHN1 or blocking p-eIF2α mediated translation with ISRIB prevents mGlu1-mediated removal of CP-AMPARs from NAc synapses and mGlu1-mediated reduction of craving. A related study is examining protein translation in the NAc of 'incubated' rats more broadly. In this study we are using rats that express Cre recombinase in either D1R or A2a/D2R medium spiny neurons to express GFP-tagged ribosomes in a cell specific manner, enabling us to isolate actively translating mRNAs from both neuronal subtypes for subsequent analysis by RNA-Seq. In support of this sequencing study, we have characterized these novel transgenic rats to ensure a high-fidelity of Cre expression in the NAc, using RNAscope and qRT-PCR.

Disclosures: A.B. Kawa: None. **E. Hwang:** None. **A.R. Nectow:** None. **H. Zhou:** None. **M. Costa-Mattioli:** None. **M.E. Wolf:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA F32 DA046141
NIDA DA049930

Title: Activity of D1 medium spiny neurons in the nucleus accumbens core is necessary for incubation of cocaine craving

Authors: *A. M. WUNSCH, J. R. FUNKE, M. E. WOLF;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: One contributing factor to relapse in individuals with substance use disorder is exposure to cues associated with drug use, which leads to intense craving and subsequent relapse. In the incubation of cocaine craving rodent model of relapse, cue-induced cocaine seeking progressively intensifies, or incubates, over the first 1-2 months of abstinence and remains elevated for at least 6 months following the last cocaine exposure. The nucleus accumbens core (NAcCo) is a critical node in the brain circuits associated with incubation. Medium spiny neurons (MSN) are the predominant neurons in the NAcCo, and can be subdivided into two populations. Neuronal activity in MSN expressing the dopamine D1 receptor (D1 MSN) drives responses to cues paired with non-contingent cocaine exposure, whereas MSN expressing the dopamine D2 receptor (D2 MSN) are not involved. To date, no studies have been performed to parse the role of these two neuron types in NAcCo in incubation. Rats expressing cre-recombinase in D1 MSN or adenosine receptor 2a (A2a) MSN (A2a is expressed exclusively in D2 MSN) received bilateral infusions of an AAV expressing cre-dependent inhibitory DREADD with a fluorescent mCherry tag (hM_4Di) or mCherry (control) in NAcCo. Two weeks later, rats underwent extended access cocaine self-administration (6h/d for 10d). Cocaine seeking tests were administered during withdrawal, during which rats nose poke for exposure to the cocaine paired cue but receive no drug. Thirty minutes prior to seeking tests on withdrawal day (WD) 50 and WD55 (when rats express maximally incubated cocaine seeking), each rat received an i.p. injection of either clozapine-N-oxide (CNO) to activate hM_4Di and reduce activity of MSN or vehicle (counterbalanced within subject design). Reducing activity of D1 MSN on WD55 attenuated drug seeking compared to vehicle injection on WD50, whereas there was no effect of the same manipulation in A2a (D2) MSN and mCherry controls. Surprisingly, activation of hM_4Di in D1 MSN with CNO injections on WD50 followed by vehicle on WD55 lead to a continued reduction in drug seeking on WD55, indicating a possible long term suppression of cocaine craving. Future studies will test how long this suppression of seeking behavior lasts following the hM4Di manipulation in D1 MSN. Furthermore, fiber photometry studies are in progress to directly assess alterations in intracellular calcium of these two neuron populations during incubated cocaine craving. Overall these results indicate that activity in D1 MSN in NAcCo is necessary for incubated cocaine seeking, and may be a therapeutic target for prevention of relapse.

Disclosures: A.M. Wunsch: None. **J.R. Funke:** None. **M.E. Wolf:** None.

Poster

P747. Cocaine: Reinforcement, Seeking, and Reinstatement I

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P747.14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA049930

NIDA T32 5T32DA007262-29

Title: Role of nucleus accumbens dopamine in the incubation of cocaine craving

Authors: *S. J. WEBER[1], A. B. KAWA[2], A. M. WUNSCH[2], M. E. WOLF[2];
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Abstract: Relapse represents a consistent clinical problem when treating individuals with Substance Use Disorder. We and others have studied this in rodents using the incubation of cocaine craving model. In this model rats undergo extended access drug self-administration where they learn to nose poke in the active port for a single infusion of cocaine paired with a 20s light cue. Next, they undergo a period of prolonged abstinence during which drug-craving increases (incubates) over time as measured by cue-induced drug seeking tests. Previous work in the Wolf lab has established glutamate plasticity, specifically the synaptic insertion of calcium permeable AMPA receptors, within the nucleus accumbens core (NAcc) as critical to the expression of incubation of cocaine craving. However, it remains unknown whether or not dopamine plays a role in incubation of cocaine craving. Dopamine is well-known for its role in motivation and salience, and would therefore be expected to contribute to a behavior reliant on increased salience of cues. In order to investigate this, we expressed dLight1.3b and implanted a fiber optic cannula in the NAcc of male and female Long Evans rats prior to cocaine self-administration (6 h/d for 10 d; 0.5 mg/kg/infusion; FR1). The dLight signal was recorded via fiber photometry during a 15-30 min cue-induced seeking test on the day immediately after self-administration ends, withdrawal day (WD) 1, and a second test late into abstinence, WD40-45. Results from an initial experiment (10 rats) show an increased dLight response following pokes into the active port compared to the inactive port. Furthermore, we observed a small trend toward an increased dLight response following active port responses in late abstinence compared to early abstinence. However, these data are preliminary and require the addition of more rats as well as saline controls. With further validation and replication this work could enhance our understanding of factors influencing relapse to cocaine.

Disclosures: S.J. Weber: None. **A.B. Kawa:** None. **A.M. Wunsch:** None. **M.E. Wolf:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA DA042584 to AGH and GVR

Title: Inhibition of PSD95-nNOS protein-protein interactions decreases morphine reward and relapse vulnerability in rats

Authors: *I. OLIVA[1], S. A. SABERI[1], C. RANGEL BARAJAS[2], V. IYER[1], K. D. BUNNER[1], Y. Y. LAI[1], P. M. KULKARNI[3], S. GARAI[3], G. A. THAKUR[3], J. D. CRYSTAL[1], G. V. REBEC[1], A. G. HOHMANN[1];
[1]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Dept. of Psychology, Indiana Univ., Indianapolis, IN; [3]Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: Glutamate-dopamine interactions through the *N*-methyl-D-aspartate receptor (NMDAR) play an unquestionable role in opioid addiction. Nevertheless, NMDAR glutamate receptor antagonists limited medical use due to their adverse side-effects. Here, we explored an alternative approach to target NMDAR signaling to reduce opiate reward and relapse through disruption of protein-protein interactions. Glutamate signaling through the NMDAR activates the enzyme neuronal nitric oxide synthase (nNOS) to produce the signaling molecule nitric oxide (NO). nNOS is tethered to NMDARs via the scaffolding protein postsynaptic density 95 kDa (PSD95). We hypothesized that the disruption of the protein-protein interaction between PSD95 and nNOS would block NMDAR-dependent NO signaling and represent a viable therapeutic route to decrease opioid reward and relapse without the unwanted side-effects of NMDAR antagonists. We used a conditioned place preference approach to evaluate the impact of two small-molecule PSD95-nNOS inhibitors – IC87201 and ZL006 – on the rewarding effects of morphine. Both IC87201 and ZL006 blocked conditioned place preference to morphine at doses that lacked intrinsic rewarding or aversive properties. Moreover, we employed intravenous morphine self-administration to examine the influence of ZL006 on the reinforcing effects of morphine. Interestingly, ZL006 did not affect morphine infusion intake, but markedly reduced lever pressing during morphine relapse after forced abstinence. Furthermore, *in vivo* fast-scan cyclic voltammetry was used to ascertain the impact of ZL006 on morphine-induced increases in dopamine efflux in the nucleus accumbens core (NAc-core) that was evoked by electrical stimulation of the medial forebrain bundle. ZL006 attenuated morphine-induced increases in dopamine efflux in the NAc-core at a dose that did not have intrinsic effects on dopamine release. Our results provide behavioral and neurochemical support for the hypothesis that inhibition of PSD95-nNOS protein-protein interactions decreases morphine reward and relapse without producing abuse liability on its own. Our studies highlight a previously unrecognized role for the use of PSD95-nNOS disruptors as a novel non-narcotic therapeutic strategy that shows promise for treating opioid addiction.

Disclosures: I. Oliva: None. **S.A. Saberi:** None. **C. Rangel Barajas:** None. **V. Iyer:** None. **K.D. Bunner:** None. **Y.Y. Lai:** None. **P.M. Kulkarni:** None. **S. Garai:** None. **G.A. Thakur:** None. **J.D. Crystal:** None. **G.V. Rebec:** None. **A.G. Hohmann:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA047102
NIDA Grant DA007287
NIEHS T32ES007254

Title: Individual differences in frustrative nonreward behavior for sucrose in rats predict motivation for fentanyl under progressive ratio

Authors: *T. E. S. VASQUEZ, P. SHAH, J. DI RE, F. LAEZZA, T. A. GREEN;
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Abstract: Abstract Frustrative nonreward is a construct in the Negative Valence Systems domain of the Research Domain Criteria (RDoC) from the US National Institute of Mental Health. An organism's response to frustrating situations (e.g. inability to obtain an expected reward) has broad implications for a variety of neuropsychiatric conditions, including substance use disorders. However, there are limited studies that investigate the role of frustration in substance use disorders. The few human studies that do investigate the role of frustration in substance use disorders highlighted that persons with substance use disorder rate higher in tests of frustration (i.e. lower frustration tolerance) and that sensitivity to frustration correlates with number of relapses. The current project developed a first of its kind rat operant behavioral model of frustrative nonreward using bar press durations as a measure of frustration-like behavior. Furthermore, the frustrative nonreward behavioral task was designed to be based loosely on the human Point Subtraction Aggression Paradigm. The present study shows that individual differences in frustrative nonreward for sucrose pellets are consistent across sessions at baseline and that the task is sensitive to reward size. More importantly, high frustrative nonreward behavior for sucrose predicts early "breaking" for intravenous fentanyl self-administration under a progressive ratio schedule demonstrating that the frustrative nonreward tasks can be used as a tool to identify rat's individual differences in frustration-like behavior during self-administration of sucrose pellets to predict a rat's drug seeking and taking prior to exposure to drug. Additionally, the "breakpoint" of an animal's behavior is also used as a measure of the relative effectiveness of the motivational properties of substance. Thus, these results support frustration/frustrative nonreward as an important aspect of motivation in substance use disorders. Furthermore, the frustrative nonreward task may be applied in future animal studies of mental health disorders as frustration is a significant component of many other neuropsychiatric conditions.

Disclosures: T.E.S. Vasquez: None. **P. Shah:** None. **J. Di Re:** None. **F. Laezza:** None. **T.A. Green:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA037207
Ancora

Title: Therapeutic Index Assessment of the M_5 Negative Allosteric Modulator VU6008667 for Attenuation of Opioid Seeking Behaviors in Rodents

Authors: L. TEAL[1], R. GOULD[2], M. BUBSER[1], M. RAGLAND[1], E. DUNCAN[1], C. W. LINDSLEY[1], *C. JONES[1];
[1]Vanderbilt Univ., NASHVILLE, TN; [2]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Accumulating data indicate that the selective inhibition of the M_5 muscarinic acetylcholine receptor subtype (mAChR) may provide an alternative therapeutic mechanism for the treatment of opioid use disorder (OUD). Previous studies by our group and others have identified the expression of M_5 mAChRs within the mesocorticolimbic dopaminergic reward circuitry and demonstrated that M_5 negative allosteric modulators (NAM) can attenuate the reinforcing effects of various substances of abuse, including cocaine, opioids and ethanol, in rats. In the present studies, we evaluated the potential dose-related effects of the highly selective M_5 NAM VU6008667 on attenuation of opioid seeking behaviors relative to the effects observed across other preclinical measures of general motor output, motivation, and autonomic and somatic motor nervous system functions in male Sprague-Dawley rats. Acute administration of VU6008667 produced dose-dependent reductions in responding for oxycodone under both fixed- and progressive-ratio schedules of reinforcement. VU6008667 also attenuated responding on the drug-associated lever in a model of opioid seeking, i.e., cue-induced responding after response extinction. Moreover, daily pretreatment of VU6008667 delayed acquisition of oxycodone self-administration in opioid naïve rats over a 21 day period. In contrast, VU6008667 had no significant effects on self-administration of sucrose pellets or rotarod performance over the dose range tested. Using the modified Irwin neurological test battery, we demonstrated that VU6008667 had no off-target effects on any autonomic or somatic motor nervous system functions at the top dose tested. Taken together, these findings suggest that negative modulation of the M_5 receptor may provide an innovative therapeutic approach with a broad therapeutic index for the treatment of OUD or prevention of misuse of prescription opioids.

Disclosures: L. Teal: A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora. **R. Gould:** A.

Employment/Salary (full or part-time):; Wake Forest School of Medicine. **M. Bubser:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora. **M. Ragland:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora. **E. Duncan:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora. **C.W. Lindsley:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora, Ono, Lundbeck and Boehringer Ingelheim. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acadia Pharmaceuticals. **C. Jones:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Ancora. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Karuna Therapeutics, Acadia Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Cumberland Emerging Technologies Scientific Advisory Board Member.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.04

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse grant UH3 DA048353
National Institute on Drug Abuse grant R01 DA047855

Title: Effects of Mitragynine on Remifentanil Self-Administration in Rats

Authors: *T. HIRANITA, J. D. ZUARTH GONZALEZ, R. C. PATEL, C. C. MAZPULE-CARRIGAN, A. PATEL, L. R. GAMEZ JIMENEZ, N. P. HO, S. OBENG, F. LEON, M.

MOTTINELLI, S. MUKHOPADHYAY, C. R. MCCURDY, L. R. MCMAHON;
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Abstract: Kratom is being developed as a pharmacotherapy to mitigate withdrawal symptoms from opioids; however, the presence of active metabolites of the primary kratom alkaloid mitragynine (MG) at mu-opioid receptors [(MORs), 7-hydoxymitragynine (7-OH-MG) and mitragynine pseudoindoxyl (MG-P)] may limit effectiveness due to their abuse potential. On the other hand, the adrenergic α_2 receptor ($A\alpha_2R$) agonist lofexidine has recently been approved as a medication to treat opioid withdrawal and several $A\alpha_2R$ antagonists have been reported to antagonize the *in vivo* effects of MG. Using an intravenous drug self-administration assay, the present study compared abuse potential of MG and its metabolites, and medications for opioid withdrawal (methadone, buprenorphine, and lofexidine), and assessed specificity of MG to antagonize the reinforcing effects of the MOR agonist remifentanil in rats. In rats self-administering remifentanil (0.1-3.2 µg/kg/injection), reference MOR agonists (methadone and buprenorphine) and the dopamine uptake inhibitor cocaine maintained self-administration responding above extinction (no injection) levels whereas lofexidine and antagonists at MOR and $A\alpha_2R$ (naltrexone and yohimbine, respectively) did not. Self-administration responding above extinction was maintained by 7-OH-MG (1.0-10 µg/kg/injection) and MGP (0.32 µg/kg/injection), but not MG (up to 3.2 mg/kg/injection) when substituted for remifentanil. Self administration of remifentanil, 7-OH-MG, and MGP was antagonized by naltrexone (3.2 mg/kg, i.p.). Self administration of remifentanil was also antagonized by methadone, buprenorphine, and lofexidine as well as MG (32 mg/kg, i.p.). The antagonizing effects of MG on remifentanil self administration were specific for remifentanil because MG (32 mg/kg, i.p.) failed to affect the dose-effect function of cocaine self-administration (0.032-1.0 mg/kg/injection). It appears that the antagonizing effects of MG on remifentanil self administration were due to the low MOR efficacy of MG relative to efficacy of remifentanil because yohimbine (3.2 mg/kg, i.p.) fully reversed the antagonizing effects of lofexidine but not of MG on remifentanil self administration. Despite the abuse potential of active metabolites of MG, these results suggest that low if any abuse potential of MG. The lack of obvious reinforcing effects of MG suggests if any low contribution of 7-OH-MG and MGP to the *in vivo* MOR activity of MG. These results also support the further development of MG as a medication with low abuse potential for opioid use disorder.

Disclosures: T. Hiranita: None. **J.D. Zuarth Gonzalez:** None. **R.C. Patel:** None. **C.C. Mazpule-Carrigan:** None. **A. Patel:** None. **L.R. Gamez Jimenez:** None. **N.P. Ho:** None. **S. Obeng:** None. **F. Leon:** None. **M. Mottinelli:** None. **S. Mukhopadhyay:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-MBRS SCORE-1SC2DA047809-02
NIH-RISE-R25GM061838

Title: Deep brain stimulation shortens extinction of persistent morphine-seeking.

Authors: *M. E. LLORET-TORRES[1], J. L. BARRETO-ESTRADA[2];
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Abstract: Opiate addiction is a chronic relapsing disorder characterized by compulsive drug use and drug seeking, regardless of negative consequences. In the United States, opiate related deaths continue to increase in what is being classified as a public health crisis. Currently, opiate addiction is treated with a combination of behavioral therapies and replacement medication. However, for a significant part of opiate addicts, these treatments remain ineffective. Deep brain stimulation (DBS) is a relatively new and novel treatment for addiction, including refractory patients under opiate abuse. Because DBS is both reversible and adjustable, it is more clinically viable than other surgical interventions. Clinical studies on DBS for addiction have shown positive results, but they tend to be limited due to the low number of participants, and some adverse effects. Therefore, pre-clinical studies to obtain more consistent and reliable results are needed. For this reason, we have previously shown that applying LF-DBS (130-Hz) to the Ventral striatum/Nucleus accumbens (VS/NAc) in male rats during sessions of morphine extinction, strengthens extinction memory. Although this result was achieved by using a partial extinction protocol, the effect of LF-DBS during full extinction remains unknown. Here, we study the effects of LF-DBS on the full extinction of morphine (5mg/kg) place preference. In addition, drug reinstatement was evaluated 24 h and 7 days after the final day of extinction. Our results show that DBS-treated animals decrease the number of extinction sessions to acquire full morphine extinction ($P < 0.001$), as compared to sham-DBS animals. Reinstatement at 7 days after the final day of extinction showed a significant reduction in drug-seeking, in animals who received LF-DBS (29%; $p < 0.005$), as compared to 57% of animals in the sham-DBS group. Our data suggest a potential therapeutic effect of LF-DBS in refractory patients of addiction by reducing both the amount of time required to fully extinguish drug-seeking, and relapse.

Disclosures: M.E. Lloret-Torres: None. **J.L. Barreto-Estrada:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA037897
NIDA Grant DA045792

Title: Glucagon-like peptide-1 receptor agonism in the interpeduncular nucleus decreases opioid reinstatement

Authors: *R. J. HERMAN, S. ZEB, K. RAGNINI, H. D. SCHMIDT;
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Abstract: Fatal opioid overdose is a leading cause of preventable death in the United States. Oxycodone is one of the most commonly abused opioid analgesics, and over one half of all opioid overdose deaths are associated with synthetic opioids including fentanyl and its analogs. Uncovering the neural mechanisms of opioid seeking will facilitate the development of novel treatments for opioid use disorder that may help to decrease or prevent opioid overdose deaths. Recent evidence indicates that glucagon-like peptide-1 receptors (GLP-1Rs) play an important role in the reinforcing and rewarding effects of drugs of abuse including opioids. Our previous studies showed that systemic administration of the GLP-1R agonist Exendin-4 (Ex-4) decreased intravenous opioid self-administration and the reinstatement of opioid-seeking behavior, an animal model of relapse, in rats. Given that GLP-1Rs are expressed throughout the brain, it is crucial to identify the GLP-1R-expressing circuits that mediate the efficacy of GLP-1R agonists in attenuating opioid seeking. GLP-1Rs are expressed at high levels in the interpeduncular nucleus (IPN), a brain region known to regulate the mesolimbic dopamine system. Based on our pilot studies with systemic Ex-4, we hypothesized that activation of GLP-1Rs in the IPN would attenuate opioid reinstatement. We trained rats to self-administer intravenous oxycodone (0.06 mg/kg/infusion) or fentanyl (1.25 μg/kg/infusion) for 21 days on a fixed-ratio 5 schedule of reinforcement. Drug taking was then extinguished by replacing the opioid solution with saline and turning off the contingent light cue. After extinction, reinstatement of opioid seeking was assessed after an acute opioid priming injection and re-exposure to conditioned light cues. Prior to each reinstatement test, rats were pre-treated with intra-IPN infusions of vehicle or Ex-4 (0.01 or 0.1 μg). We showed that intra-IPN infusions of Ex-4 dose-dependently decreased drug + cue-induced reinstatement of fentanyl- and oxycodone-seeking behaviors. Importantly, our preliminary data indicate that IPN Ex-4 inhibits opioid seeking at doses that do not affect 24 hr body weight and water intake. Additionally, we found that GLP-1Rs are expressed on GABAergic neurons on the IPN, providing a potential mechanism for Ex-4's effect on opioid seeking. Overall, these results support a functional role of IPN GLP-1R activation in Ex-4's effect on opioid seeking and support the use of GLP-1R agonists as a potential treatment for opioid use disorders.

Disclosures: R.J. Herman: None. **S. Zeb:** None. **K. Ragnini:** None. **H.D. Schmidt:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of sucrose exposure during the adolescent period on oxycodone self-administration

Authors: J. WATSON[1], M. CRAWFORD[2], M. HOCHSTETLER[1], C. ALONSO[1], L. EVANS[1], *A. GANCARZ-KAUSCH[1];
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Abstract: Sugar is a ubiquitous naturally occurring substance that holds strong hedonic value, and many adolescents exceed the U.S. Dietary Guidelines for sugar consumption (Ervin, R.B. et al., 2012). The adolescent period is a critical time of development and is also a time where adolescents face an increased risk for substance abuse (Winstanley & Stover, 2019). Furthermore, ample evidence suggests that drug use during the adolescent period increases future vulnerability to drug abuse, including opioids (Windisch & Kreek, 2020). However, the impact that natural rewards, like sugar, have on adult behavior is less understood. Previous work from our lab indicates that exposure to sucrose in adolescence increases vulnerability to cocaine and alcohol self-administration in adult male and female rats. The purpose of this study was to investigate if the effects of adolescence sucrose exposure generalize to self-administration of oxycodone in adulthood. To test this, adolescent male rats (PND 28-42) were given noncontingent access to either 10% sucrose solution or water. Exposure sessions were 30 min in duration and 100 microliters of solution (sucrose or water) was presented according to a variable time 1 min schedule of reinforcement for 15 days. After this phase, rats were placed back in the colony room with food and water available ad libitum until adulthood. Once the rats reached adulthood (PND 60), they were tested for oxycodone self-administration for 3-hour sessions across 7 days. Active responses resulted in the delivery of 0.15 mg/kg/infusion oxycodone according to a FR1 schedule of reinforcement. Rats exposed to sucrose during the adolescent period earned significantly more infusions of oxycodone compared to water-exposed controls during self-administration testing. Furthermore, rats exposed to sucrose during the adolescent period show significantly greater preference for oxycodone, as measured by the relative frequency of active responses, compared to water controls. Taken together, these findings have important implications to the human condition, suggesting exposure to sucrose during the adolescent period may increase susceptibility to non-natural rewards in adulthood.

Disclosures: J. Watson: None. **M. Crawford:** None. **M. Hochstetler:** None. **C. Alonso:** None. **L. Evans:** None. **A. Gancarz-Kausch:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA U01DA043098
Office of Naval Research (ONR) 00014-19-1-2149
The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium
NIDA T32DA07268

Title: Intravenous self-administration of heroin in an animal model of internalizing vs externalizing temperament

Authors: *M. A. EMERY[1], A. PARSEGIAN[1], P. M. MARAS[1], E. K. HEBDA-BAUER[1], S. B. FLAGEL[1,2], S. J. WATSON, Jr.[1,2], H. AKIL[1,2];
[1]Michigan Neurosci. Inst., [2]Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: In humans, only a small portion of those who experiment with recreational drug use transition to a substance use disorder (SUD). Multiple factors are thought to underlie this susceptibility, including differences in temperament. Those with externalizing temperaments are hypothesized to approach drugs of abuse from a sensation-seeking pathway, while those with internalizing temperaments appear less likely to approach drugs of abuse for purely recreational reasons but rather in response to other triggers such as psychosocial stress. Our lab has used a selective breeding strategy based on degree of exploratory locomotion in a novel environment to derive two lines of rats with distinct behavioral and neurobiological phenotypes, termed selectively-bred high-responders (bHRs) and low-responders (bLRs). We have previously shown that temperamental differences between these rats determines their propensity to seek and take psychostimulant drugs. Specifically, bHRs, who display a sensation-seeking phenotype, demonstrate a higher basal propensity to take drugs; whereas bLRs appear less susceptible at baseline but will seek and take drugs in response to psychosocial stress. While we have shown that bHRs and bLRs have biological differences in endogenous opioid system, such as differential expression levels of mu opioid receptor, we had not examined bHR/bLR differences in intravenous self-administration of opioids. Here, we used a free access self-administration paradigm of diacetylmorphine (heroin) to determine whether these individual differences impact self-administration of opioids. We observed the expected phenotype differences at baseline where bLRs seek and take less drug, replicating the pattern seen with psychostimulants. Interestingly, a small but potentially important sex x genotype interaction has emerged where female bHRs sought and took more heroin than males, a pattern not observed for bLRs. Future experiments will elaborate on this sex difference, as well as exploring the impact of stress on heroin self-administration. These findings contribute to our understanding of the impact of individual differences as well as a potential interaction between biological sex and temperament upon the trajectory of opioid addiction development.

Disclosures: M.A. Emery: None. **A. Parsegian:** None. **P.M. Maras:** None. **E.K. Hebda-Bauer:** None. **S.B. Flagel:** None. **S.J. Watson:** None. **H. Akil:** None.

Poster

P756. Opioids: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P756.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA044980

Title: Stressor Controllability Provides Resilience Against Oxycodone Seeking in a Forced Abstinence Incubation of Drug Craving Model

Authors: *K. T. BROWN[1], K. A. SILETTI[2], M. C. HILLMAN[2], E. C. WARNOCK[2], M. V. BARATTA[2], M. P. SADDORIS[2];
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Abstract: Psychological stress is a critical barrier to the maintenance of abstinence among individuals with opioid use disorder. A substantial pre-clinical literature has modeled the effects of stress on drug-seeking and has identified neural circuit and neurochemical consequences of stress-induced drug seeking. The perception of control over a stressor (i.e., stressor controllability) is an important mitigating factor that determines the behavioral and neurobiological consequences of stress. Despite its known importance, stressor controllability has not been modeled in pre-clinical models of opioid drug-seeking. The present study uses a well-characterized model of stressor controllability to examine its behavioral consequences in a forced abstinence model of incubation of craving. Sprague-Dawley rats self-administered intravenous oxycodone hydrochloride (Oxy) (100 µg/kg/infusion) on a fixed-ratio 1 schedule of reinforcement during 14 daily 6-hour sessions. During these sessions, presses on an active lever delivered i.v. Oxy along with illumination of a cue light for 5 sec over the lever; presses on the inactive lever illuminated the above cue light, but did not deliver any drug. Rats then underwent a 30-minute drug-associated cue-reinforced drug seeking session on day 1 of forced abstinence from Oxy and a 1-hour drug-seeking session on day 15 of forced abstinence. 48 hours before the day 15 session, rats were randomly assigned to a home-cage control (HC) group, escapable stress (ES) session, or inescapable stress (IS) session. Rats in the escapable stress group underwent a 120-minute session during which 100 1 mA tail-shocks were administered. Rats in the ES condition controlled the duration of the tail-shock by turning a wheel on a FR4 schedule. Rats in the IS group were yoked to the ES group and experienced identical duration and intensity of tail-shock as the yoked subjects in the ES group. Importantly, the IS group was not able to control the stressor via wheel turns. Rats in the ES, but not IS, group pressed significantly less on the

cue-reinforced, previously drug-associated lever than the HC group during the day 15 drug-seeking session. Future studies will assess the effects of manipulating the medial prefrontal cortex, a brain region previously found to underlie protective effects of stressor controllability, during stress to better understand neural circuitry involved in ES mediated reductions in Oxy seeking. These data suggest that the experience of stressor controllability reduces drug-seeking and may provide a pre-clinical model to investigate neurobiological mechanisms contributing to the protective effects of stressor controllability.

Disclosures: K.T. Brown: None. **K.A. Siletti:** None. **M.C. Hillman:** None. **E.C. Warnock:** None. **M.V. Baratta:** None. **M.P. Saddoris:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.01

Topic: H.01. Attention

Support: PHS Grant RO1DA045063
P50NS091856

Title: Cognitive-motivational traits indexed by sign- vs. goal-tracking extend to cortico-striatal glutamatergic signaling and complex movement control

Authors: *C. AVILA[1], M. SARTER[2];
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Abstract: Complex movements involve the utilization of task cues to guide movement selection and sequencing. We previously demonstrated that such detection of cues necessitates cortical cholinergic signaling, and that rats mimicking the cortical cholinergic and striatal dopaminergic losses that characterizes Parkinsonian fallers likewise are prone to falls. Indeed, rats with only cortical cholinergic signaling deficiencies exhibit complex movement control deficits. We also reported that sign-tracking rats (STs), that is, rats which are prone to readily approach and utilize drug cues, are characterized by poor attentional performance, relative to their counterparts, the goal-trackers (GTs), and that this is mediated by attenuated cortical cholinergic activity. Predictably, STs are less efficient in traversing dynamic surfaces than GTs. Here, we measured real-time dorsomedial striatal glutamate (GLU) signaling, using amperometry and biosensors, to test the hypotheses that; a) cues guiding movement shifts are encoded by cortico-striatal GLU and; b) that GLU activity is attenuated in STs when compared with GTs. STs and GTs were trained to perform a cue-triggered turning task (CTTT), requiring rats walking on a treadmill to utilize turn and stop cues. GLU bursts were selectively evoked by the turn cue in all rats.

However, in trials in which the cue was not utilized ("missed turns"), glutamate bursts were attenuated GTs but not STs. These findings indicate that only cues triggering a shift in movement programming - turns - evoke striatal GLU, and that GLU bursts predict trial outcome in GTs but not in STs. Performance did not differ between STs and GTs, consistent with prior evidence indicating that instructional cues are learned similarly by both phenotypes and that differences emerge specifically on tests that probe motivational value. Such an "insulation" of the striatum from cortical influence may essentially contribute to the vulnerability of STs for addiction-like behavior. Ongoing experiments are designed to demonstrate that cue-locked glutamatergic signaling originates specifically from cortico-striatal projections and that the indiscriminate striatal glutamate signaling seen in STs can be reproduced in GTs by cortical cholinergic deafferentation.

Disclosures: C. Avila: None. **M. Sarter:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.02

Topic: H.01. Attention

Support: PHS Grant RO1DA045063

Title: Vulnerable for addiction-like behavior: disrupted cholinergic signaling and exaggerated neuroimmune response in sign-tracking rats

Authors: *H. CARMON[1], V. PARIKH[2], E. HALEY[2], N. TRONSON[1], M. SARTER[1];
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Abstract: Some rats (sign-trackers; STs) are prone to attribute incentive salience to reward cues, which can manifest as a propensity to approach and contact Pavlovian cues, and for addiction-like behavior. STs also exhibit poor attentional performance, relative to their counterparts, the goal-trackers (GTs), mediated by attenuated cholinergic activity. Poor cholinergic-attentional control contributes to the propensity of STs to approach and utilize Pavlovian drug cues. In STs, increases in neuronal activity fail to translocate choline transporters (CHTs) into synaptosomal plasma membrane, thereby limiting the capacity of cholinergic terminals to sustain cholinergic activity. Here we investigated post-translational modifications responsible for disrupted CHT trafficking in STs, and the hypothesis that attenuated cholinergic activity in STs causes exaggerated (neuro)immune responses. We determined levels of ubiquitinylated CHTs because ubiquitin-mediated processes can account for attenuated externalization of intracellular CHTs, ranging from proteolysis to enhanced internalization or alternative compartmentalization. The proportion of ubiquitinylated CHTs, extracted from cortex, was robustly higher in STs than in

GTs, with no overlap in the data from the two phenotypes. Moreover, modified CHTs located in intracellular domains, but not in synaptosomal plasma membrane, completely accounted for the phenotype-specific ubiquitylation levels. Ongoing experiments suggest elevated spleen levels of pro-inflammatory cytokines in unchallenged STs. Following a systemic lipopolysaccharide (LPS) immune challenge, brain levels of certain cytokines were elevated as a complex function of LPS dose, phenotype, and sex. Moreover, following LPS challenge, ubiquitinylation levels of CHTs from the cortex were drastically increased in GTs, but not STs, suggesting that in unchallenged STs, ubiquitinylated CHTs already are at maximum levels and unresponsive to an additional immune challenge. Together, this evidence supports potentially escalating, bidirectional interactions between disrupted cholinergic signaling and elevated (neuro)immune responses as an integral component of the cognitive-motivational trait indexed by sign-tracking.

Disclosures: H. Carmon: None. **V. Parikh:** None. **E. Haley:** None. **N. Tronson:** None. **M. Sarter:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.03

Topic: H.01. Attention

Title: The acetylcholinesterase inhibitor Donepezil and the M_1 positive allosteric modulator VU0453595 produce distinct dose dependent pro-cognitive profiles in monkeys

Authors: *S.-A. HASSANI[1], J. RUSSELL[2], S. LENDOR[3], A. NEUMANN[2], K. SINHA ROY[3], K. BANAIE BOROUJENI[1], J. PAWLISZYN[3], K. L. HOFFMAN[1], C. K. JONES[2], T. WOMELSDORF[1];
[1]Dept. of Psychology, [2]Vanderbilt Univ., Nashville, TN; [3]Univ. of Waterloo, Waterloo, ON, Canada

Abstract: The cholinergic system consists of nicotinic and muscarinic receptors and is impaired in numerous neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease (AD). Donepezil, an acetylcholinesterase inhibitor, is one of the few FDA approved pro-cognitive drugs for the treatment of dementia in AD. Previous animal studies have demonstrated that donepezil can enhance multiple cognitive domains, including learning, working memory, and attention functions. However, the therapeutic potential of Donepezil is limited by peripheral cholinergically mediated dose-limiting adverse effects and by differences in the maximally effective dose for different cognitive domains. Here, we addressed these limitations by first determining the optimal dose range of Donepezil for enhancing multiple cognitive domains with a novel cognitive assessment protocol for rhesus monkeys, and secondly by evaluating the selective M_1 muscarinic receptor positive allosteric modulator (PAM) VU0453595 under the

same protocol to determine whether this mechanism would provide a broader therapeutic index for cognitive enhancement. We evaluated the potential dose-related effects of Donepezil and VU0453595 in four male adult (6-9 years old) rhesus macaques (*Macaca mulatta*) performing a visual search task for assessing interference control (attention), arousal, and impulsivity, and a feature learning task for assessing cognitive flexibility, reward sensitivity and perseveration tendencies. We found that Donepezil enhanced cognitive flexibility and interference control, contingent on task difficulty. The dose at which Donepezil exerted its maximal pro-cognitive effects differed based on task specifics. These enhancements were accompanied by slower reaction times and peripheral cholinergic side effects at the maximally cognitively-effective doses. We also utilized solid phase micro-extraction (SPME) microprobes to validate that Donepezil was available in the prefrontal cortex and striatum, highly relevant brain regions for the performed tasks. In contrast to Donepezil, VU0453595 improved both reaction times and accuracy at doses with no adverse effects, producing a distinct profile of pro-cognitive effects.In summary, Donepezil and the M_1 PAM VU0453595 produced distinct dose-dependent pro-cognitive effects across two cognitive domains. Current findings indicate that our novel multi-task cognitive assessment protocol provides a versatile framework for evaluating pharmacological influences on multiple cognitive domains in the rhesus monkey.

Disclosures: S. Hassani: None. **J. Russell:** None. **S. Lendor:** None. **A. Neumann:** None. **K. Sinha Roy:** None. **K. Banaie Boroujeni:** None. **J. Pawliszyn:** None. **K.L. Hoffman:** None. **C.K. Jones:** 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.; An inventor on patents protecting allosteric modulators of muscarinic acetylcholine receptors; receives royalties from Acadia Pharmaceuticals and research support from Ancora. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owns personally-acquired stock in Karuna Therapeutics. **T. Womelsdorf:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.04

Topic: H.01. Attention

Support: NIH Grant R01GM126155

Title: Recovery of sustained attention following general anesthesia in rats

Authors: *K. VINCENT, E. ZHANG, R. KATO, A. CHO, O. MOODY, K. SOLT; Anesthesia, Critical Care, and Pain Med., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Despite the ubiquity of general anesthesia in medical practice, our understanding of how cognition is restored following anesthetic-induced breaks in consciousness is surprisingly limited. Of particular importance is the recovery of attentional processes. Attentional deficits are a hallmark of postoperative delirium, a serious and common postoperative complication in elderly patients. Establishing valid and relevant models to investigate these impairments preclinically will be critical for understanding their underlying neural mechanisms. Here we implement the 5-choice serial reaction time task (5-CSRTT), an analog to human continuous performance tasks, to assess the real-time recovery of sustained attention in rats following general anesthesia. In the task, rats attend a 5-windowed screen for the brief presentation of a white square. Selecting the correct window within 5 seconds is rewarded with a food pellet while incorrect selections or missed responses are punished with a time-out. During each session we tracked both the proportion of correct responses (accuracy) and proportion of missed responses (omissions) over time. We assessed attention recovery trajectories from isoflurane (2%, one hour), sevoflurane (3%, 20 minutes), propofol (10 mg/kg I.V. bolus), ketamine (50 mg/kg over 10 minutes I.V.), and dexmedetomidine (20 and 35 μg/kg over 10 minutes I.V.) for up to three hours following the return of the righting reflex (RORR), a measure of consciousness. We classified rats as having recovered accuracy performance when four of their last five responses were correct, and as having recovered low omission performance when they missed one or fewer of their last five trials. Following isoflurane, sevoflurane, and propofol anesthesia the majority (63-88%) of rats recover both accuracy and low omission performance within an hour of RORR. Following ketamine, accuracy performance in most (63%) rats recovers by two hours, but low omission performance recovers in only a minority (32%) of rats within three hours. Finally, following either high or low doses of dexmedetomidine, few rats (25-32%) recover accuracy performance within three hours, and even fewer (0-13%) recover low omission performance within three hours. Regardless of the anesthetic, RORR latency is not correlated with 5-CSRTT performance, which suggests that recovery of neurocognitive function cannot be inferred from changes in levels of consciousness. Overall, we demonstrate that the 5-CSRTT can be exploited to track real-time recovery of sustained attention in trained rats following anesthetic emergence to capture clinically relevant features of neurocognitive function.

Disclosures: K. Vincent: None. **E. Zhang:** None. **R. Kato:** None. **A. Cho:** None. **O. Moody:** None. **K. Solt:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.05

Topic: H.01. Attention

Support: NIH R34NS111653

Startup funds from the Johns Hopkins University

Title: Role of the caudal patch of the secondary motor cortex (cM2) in visual decision-making in mice

Authors: *W.-K. YOU, S. P. MYSORE;
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Abstract: The caudal patch of the secondary motor cortex (cM2) sits at a unique position in the motor network of the mouse brain. Unlike the rest of the motor cortices, which connect profoundly to somatosensory cortices and are involved in the control of body movements, the cM2 connects mainly to visual cortices and has been documented to be responsive to visual stimuli. Electrically stimulating this area causes saccade-like eye movements, while optogenetically inactivating this area prohibits contraversive eye movements in mice. These results suggest that cM2 may be a key area in the mouse oculomotor network. Nonetheless, the visual response properties of cM2 neurons remain largely uncharacterized, and the necessity of cM2 in visual decision-making has not yet been examined. Here, with *in vivo* calcium imaging using the Quartet microendoscope (Neurescence Inc.), we characterize the properties of cM2 neurons in response to visual stimuli. We also examine the necessity of cM2 in visual decision-making by chemogenetically inactivating it in freely behaving mice engaged in an orientation discrimination task. Our results can reveal novel properties and function of this visuomotor nexus within mouse M2.

Disclosures: W. You: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurescence Inc. **S.P. Mysore:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurescence Inc..

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.06

Topic: H.01. Attention

Support: R01 NS104923

Title: Coherent beta (15-30 Hz) activity inhibits exogenous selection in prefrontal cortex (PFC)

Authors: *A. DUBEY[1], D. A. MARKOWITZ[1], B. PESARAN[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: Visual selection is an attentional mechanism with which we decide what to see and act upon. In primates, at least two distinct attentional processes, sensory-driven exogenous (“bottom-

up") and goal-driven endogenous ("top-down") attention, drive visual selection for eye movement decisions. Behavioral evidence indicates that exogenous processing is faster and deployed earlier in time, endogenous processing is slower and occurs later in time and exogenous and endogenous attention linearly combine in time to guide visual selection. While the neural mechanisms of attentional selection remain unclear, they must support the dynamic interplay between endogenous and exogenous attentional processing. We investigated the neural mechanisms of attentional selection in the lateral prefrontal cortex (PFC) which is known to receive inputs from both visual-driven exogenous and reward-driven endogenous processes. We simultaneously recorded spiking and field potential activity from a semi-chronic 32-channel drive in two monkeys, during a two-target, eye-movement task. The task dissociated exogenous and endogenous processes by simultaneously manipulating relative luminance and reward differences. We found that the firing rate of individual visual-movement (VM) neurons (and not visual and movement neurons) encodes both exogenous and endogenous selection. Consistent with behavioral performance, selection times estimated from neural firing were faster for exogenous than endogenous selection (mean ST:88 vs 163ms). Further, neural activity during the pre-target baseline period reflected the action of a coherent beta (15-30 Hz) network. Spike-field coherence was significantly stronger during high-amplitude beta events than low-amplitude beta events. Importantly, increased pre-target beta power showed a decrease in post-target firing activity for exogenous selection but not endogenous selection. These results provide neural evidence of distinct exogenous and endogenous attentional mechanisms and suggest that inhibitory mechanisms due to coherent beta activity specifically modulate exogenous attentional selection.

Disclosures: A. Dubey: None. **D.A. Markowitz:** None. **B. Pesaran:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.07

Topic: H.01. Attention

Support: RMH Neuroscience

Title: Mouse hippocampus neural ensembles are tuned near criticality during a cognition task

Authors: F. HABIBOLLAHI[1], J. WINNE[2], R. N. LEAO[3], A. N. BURKITT[4], *C. FRENCH[1]; [1]Univ. of Melbourne, Melbourne, Australia; [2]Developmental Genet. Unit, Federal Univ. of the Rio Grande do Norte, Brazil, Rio Grande de Norte, Brazil; [3]Brain Inst., UFRN, Natal, Brazil; [4]Dept. of Biomed. Engin., The Univ. of Melbourne, Melbourne, Australia

Abstract: Background Dynamical systems exhibit transitions between ordered and disordered states and “criticality” occurs when the system lies at the borderline between these states at which the input is neither strongly damped nor excessively amplified. In this study we report strong evidence of criticality properties in freely behaving animals using widefield neural calcium imaging.
Methods 15-minute episodes of widefield calcium imaging from several hundred hippocampal CA1 neurons using “Miniscopes” in 6 freely behaving mice were used to study criticality measures during the cognitive task of novel object recognition (NOR). Neural avalanches were identified using another thresholding step on the ensemble activity and four independent criticality measures were measured: power-law distribution, deviation from criticality (DCC), shape collapse, and branching ratio (BR).
Results The 4 criticality parameters were measured in 7 recording sessions of approximately 300 detected units, each of 15 mins, from 6 freely behaving mice. These consist of 3 inactive sessions and 4 sessions during NOR. We find that while the hippocampus neural network exhibits characteristics of a near-critical system at rest (power law expands less than 2 orders of magnitude, DCC = 0.770±0.416, shape collapse error = 0.260±0.018, BR = 0.958±0.004 in mean±SE), the network activity shifts closer to a critical state when the mice engaged in cognitive tasks (power law expanded at least 2 orders of magnitude, DCC = 0.291±0.095, shape collapse error = 0.090±0.033, BR = 0.979±0.018 in mean±SE).
Conclusions Our results utilizing a novel calcium imaging technique and analysing 4 independent measures of criticality in the hippocampus neural during a cognitive task, for the first time , suggest that switching from inactivity to a cognitively active state modulates the network’s distance from criticality; meaning it decreases DCC and shape collapse error while increasing BR. Our findings imply that being tuned near criticality is potentially the optimal state for the brain network while engaged in a cognitive task given at criticality, the dynamical range and the information content and transmission are maximized.

Disclosures: F. Habibollahi: None. **J. Winne:** None. **R.N. Leao:** None. **A.N. Burkitt:** None. **C. French:** None.

Poster

P772. Mechanisms of Attention: Animal Models II

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P772.08

Topic: H.05. Working Memory

Support: T32 HD041921-18
NARSAD Young Investigator Award 28303
R01 HL142752

Title: Maternal sleep disordered breathing during pregnancy is a previously unrecognized facilitator of autism-relevant neuronal and behavioral aberrations in the offspring.

Authors: *A. VANDERPLOW[1], B. A. KERMATH[1], C. R. BERNHARDT[1], K. T. GUMS[1], A. S. ROOPRA[2], T. L. BAKER[1], J. J. WATTERS[1], M. E. CAHILL[1];
[1]Comparative Biosci., [2]Neurosci., The Univ. of Wisconsin-Madison, Madison, WI

Abstract: Sleep disordered breathing (SDB) is characterized by recurring breathing cessations during sleep, causing intermittent hypoxia (oxygen deprivation) often several hundred times per night. Obstructive sleep apnea is the most common form of SDB in pregnancy, and confers risk for detrimental maternal-fetal outcomes. Although many of the maternal risk factors for the onset SDB during pregnancy and the development of autism spectrum disorders (ASD) in her offspring are highly overlapping (e.g., maternal obesity, advanced maternal age), no studies to date have empirically investigated the potential link between these conditions. We subjected pregnant rat dams to intermittent hypoxia or normoxia (GIH and GNX, respectively) from gestation day 10-21 to investigate the consequences of maternal SDB on offspring neural function. We found that male GIH offspring exhibited deficits in several behaviors which were accompanied by increased pyramidal neuron dendritic spine density in the medial prefrontal cortex (PFC). These effects appear to arise from a disruption of normal developmental synaptic pruning late in childhood and persisting into early adolescence. The mTOR kinase signal transduction pathway has emerged as a critical regulator of dendritic spine formation, and dysregulation of key components of this pathway are implicated in the etiology of the increased cortical dendritic spine density of ASD. Our data in prefrontal cortical neuronal synaptic fractions from male GIH offspring indicate that mTOR activity exhibits a biphasic pattern: mTOR signaling is increased in juvenile offspring, yet decreased in adult offspring, relative to male GNX offspring. RNA-sequencing data from adult male GIH microglia isolated from the PFC also identified increased mTOR-dependent gene expression, suggestive of mTOR pathway dysregulation in both neurons and microglia. Together, these data indicate that excessive neuronal mTOR signaling could contribute to the lack of spine pruning in juvenile male GIH offspring, while the subsequent decrease in neuronal mTOR signaling at later stages of development could serve a compensatory role to mitigate further surges in spine numbers. Enhanced microglial TOR signaling may also contribute to increased spine numbers by reducing the natural ability of microglia to prune synapses. Ongoing studies are aimed at determining whether reducing mTOR activity in juvenile GIH offspring ameliorates the behavioral, neuronal, and microglial abnormalities in adolescent GIH offspring.

Disclosures: A. Vanderplow: None. **B.A. Kermath:** None. **C.R. Bernhardt:** None. **K.T. Gums:** None. **A.S. Roopra:** None. **T.L. Baker:** None. **J.J. Watters:** None. **M.E. Cahill:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.01

Topic: H.03. Decision Making

Support: National Institutes of Health Grant K01-DA-036659-01
Busch Biomedical Foundation
Whitehall Foundation

Title: Estimating subjective value and decoding choice intent from neural populations in primate orbitofrontal cortex

Authors: *V. B. MCGINTY, S. M. LUPKIN;
Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ. - Newark, Newark, NJ

Abstract: In neuroeconomics, one of the key ways of identifying decision mechanisms is to show a trial-to-trial relationship between neural variability and decision variability. In a classic example, Britten et al. (1996) showed that the activity of motion-sensitive neurons could explain variability in motion judgements, even over trials in which the same noisy motion stimulus was repeated many times. Finding this fine-grained neural-behavioral link is important because it provides insight into how neural activity is read out by downstream circuits, and (along with other findings) evinces the causal role of neural activity for behavior. For value-based decisions, neurons in the primate orbitofrontal cortex (OFC) are thought to have such a causal role. Specifically, they are thought to shape decision outcomes by encoding economic value; however, a robust link between variability in OFC value coding and variability in value-based decisions has yet to be found. In this study, we report such a link for the first time. Macaque monkeys performed a decision task in which they selected between two visual targets on the basis of reward value (number of drops of juice). Critically, many trials offered choices between targets of similar or equal value, producing substantial inter-trial variability in decision outcomes. Neural recordings were obtained from populations of OFC neurons (~45 cells per session) using acutely placed linear arrays. To link decision variability to neural variability, we used a regularized linear encoder/decoder that took advantage of the simultaneous nature of the neural recordings. First, a set of training trials was used to assign to each cell a weight which indicated that cell's unique contribution to representing the target values (encoding); then, over a set of held out test trials, we found the weighted sum of spiking across all cells, which we took as an estimate of the subjective values of the targets on each trial (decoding). Finally, the subjective value estimates were used to classify decision outcomes (i.e. to infer decision intent) in the test trials. With this population-level decoder, it was possible to predict decision outcomes with far higher accuracy than with a decoder that used single cells individually. Examination of the encoding/decoding weight vectors indicated that OFC value representations were nearly orthogonal at the population level, and were therefore linearly separable by downstream circuits. Thus, these findings provide an essential piece of evidence to support the role of OFC in value-based choice, as well as specific insights into the mechanisms by which OFC value representations inform economic decisions.

Disclosures: V.B. McGinty: None. **S.M. Lupkin:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.02

Topic: H.03. Decision Making

Support: NIH Grant T32MH019524
Training Program in Computational Neuroscience 5T90DA043219
K99/R00 Pathway to Independence Award (5R00MH111926-05)
Alfred P. Sloan Fellowship
Klingenstein-Simons Fellowship
NIH Director's New Innovator Award (DP2MH126376)
R01 (1R01MH125571-01)

Title: Lateral orbitofrontal cortex is required for inferring hidden task states

Authors: *S. SCHIERECK, A. MAH, C. CONSTANTINOPLE;
New York Univ., New York, NY

Abstract: A principle of modern decision theories is reference dependence, in which the subjective value of a reward is computed relative to an internal reference point that is thought to reflect outcome expectations. Previous work has often assumed that reference points are retrospective, deriving directly from past experiences. Here, we show that rats can use inference to compute and switch between multiple reference points, and that the lateral orbitofrontal cortex (lOFC) is required for this capacity. We have designed a behavioral paradigm with partially-observable states (blocks of trials offering small or large rewards). Rats must determine how long to wait for uncertain rewards, providing an explicit behavioral readout of a rat's subjective value for rewards. Rats' wait times are sensitive to the reward volumes and also the block structure: rats tend to wait longer for the same volume of water reward in blocks of trials with a low average expected reward compared to blocks of trials with a high average expected reward. Behavioral analysis suggests that they are using inference, as opposed to retrospection, to adjust their reference points based on the inferred block. To assess the role of lOFC in this task, we performed whole-session inactivation of lOFC using bilateral muscimol infusions (n=8 rats). We found that modulation of wait-time by reward volume is not impaired by lOFC inactivation, suggesting that this region is not required for computing the subjective value for different reward volumes, per se, or for time estimation. However, modulation of wait-time by reward block was dramatically reduced, suggesting that lOFC was required for rats to infer the hidden block. We present results from behavioral modeling, comparing models that instantiate retrospective or inferential strategies, to characterize the precise nature of the behavioral deficit in greater detail. Additionally, we are performing chronic freely-behaving neuropixels recordings to understand

when and how task-relevant variables including the current reward block, offered reward volume, and delay to reward are encoded in lOFC in this task.

Disclosures: S. Schiereck: None. **A. Mah:** None. **C. Constantinople:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.03

Topic: H.03. Decision Making

Support: NIH/NINDS/ 2RF1NS023945-28

Title: Basal forebrain cholinergic projections to reciprocally connected orbitofrontal and secondary visual cortex.

Authors: *C. CHAVEZ, L. ZABORSZKY;
Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: The basal forebrain (BF) cholinergic system provides acetylcholine (ACh) throughout the cerebral cortex and has been implicated in cortical activation, attention, sensory processing, learning, and memory. Despite innervating the entire neocortex, studies have found selective ACh release in distinct cortical areas, likely supported by the neuroanatomical findings that cholinergic cells within the BF project to specific cortical targets. Together, this suggests that the BF may be involved in modulating cortical function in particular cortical regions rather than in a global manner. Recently, using electrophysiology we showed that neurons in sub-regions of the BF are dynamically recruited during various behavioral epochs of a visual discrimination task (Gombkoto et al., 2021). Additionally, we found that specific BF cholinergic cells may be involved in modulating gamma coherence between reciprocally connected cortical regions. We hypothesize that interconnected cortical regions may share overlapping cholinergic projection neurons within specific sub-regions of the BF that underlie the increased corticocortical coherence observed during behavior. Using dual retrograde tracer injections, we targeted both the secondary visual (V2) and orbitofrontal cortex (OFC) which our study found is co-modulated by BF cholinergic activity and is known to be reciprocally connected. Adult Chat/Cre negative rats were injected with Fluoro-Gold (Fluorochrome) and Fast Blue (Polysciences, Inc.) retrograde tracers into V2 and OFC respectively. After a 1-week survival period, the brain tissue was collected and processed for observation using a fluorescence microscope (Axioskop, Zeiss). Tissue sections were evaluated every 200µM for fluorescent labeling using Neurolucida software (MBF Bioscience). Preliminary data suggests that a subpopulation of OFC projecting and V2 projecting BF neurons overlap in their localization within the BF. Further, preliminary data utilizing viral tracing methods in Chat/Cre positive rats identified several cholinergic cells

double labeled for both the OFC and V2 projections, suggesting that single cells within the BF may collateralize to project to the interconnected cortical targets. This preliminary data along with our previous electrophysiological data supports the hypothesis that interconnected cortical regions share overlapping subpopulations of BF projection cells and they may underlie the co-modulation observed in these cortical targets during behavior.

Disclosures: C. Chavez: None. **L. Zaborszky:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.04

Topic: H.03. Decision Making

Title: Aspiration lesions of OFC disrupt cholinergic innervation of the prefrontal cortex in the rhesus macaque

Authors: *B. E. HINES, M. A. ELDRIDGE, E. A. MURRAY;
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Abstract: Previous studies have shown that monkeys with bilateral aspiration lesions of orbitofrontal cortex (OFC) are impaired in serial object reversal learning, whereas monkeys with excitotoxic lesions of the same region are not (PH Rudebeck et al., *Nat. Neurosci*., 2013). This discrepancy indicates that aspiration lesions of OFC affect the brain differently than excitotoxic lesions of OFC, presumably by disrupting fibers of passage, i.e., axons passing nearby or through the OFC en route to other regions of the brain. Here, we sought to determine how aspiration lesions of OFC impact the distribution of cholinergic fibers in other regions of prefrontal cortex. Three rhesus monkeys received unilateral aspiration lesions of OFC. Monkeys were sacrificed 5, 6 and 56 weeks after surgery, their brains were fixed, slices were cut at 40 microns, and Acetylcholine-esterase (AChE) immunohistochemistry was performed. AChE distribution was measured as a proxy for cholinergic fiber distribution, quantified, and comparisons between the OFC-lesioned and intact hemispheres were performed. Densitometry analysis was used to quantify the strength of cholinergic fibers in five sections of each hemisphere per monkey. We examined anterior cingulate cortex because it receives inputs from many temporal lobe structures via projections that run through or nearby the OFC, in addition to receiving inputs from cholinergic neurons in the basal forebrain. As a negative control, we examined the striatum. Aspiration removals of OFC significantly reduced the density of acetylcholine fibers in the anterior cingulate cortex, but not our control site in the striatum. The significant reduction in the density of stained fibers in the ACC of hemispheres with aspiration lesions of OFC indicates that cholinergic projections travel through the OFC en route to the ACC, consistent with earlier

reports (Kitt et al., *Brain Res*. 1987). These results suggest a possible explanation for the differential effects on behavior of aspiration versus excitotoxic lesions of OFC.

Disclosures: B.E. Hines: None. **M.A. Eldridge:** None. **E.A. Murray:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.05

Topic: H.03. Decision Making

Support: NIH Grant MH084970
NIH Grant MH118237

Title: Medial orbitofrontal cortex outputs facilitate risk assessment and govern reward-related circuits in an age-dependent manner

Authors: *M. K. LOH, N. C. FERRARA, J. M. TORRES, J. A. ROSENKRANZ;
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Abstract: Risk assessment precedes appropriate decision-making, which is crucial for survival but riskier during adolescence. The developing circuits that underlie risk assessment are not well understood but can further our understanding of high-risk behaviors. The nucleus accumbens (NAc) integrates excitatory inputs from both cortical and limbic structures, which are communicated to downstream motor regions. As inputs from cortical and limbic structures carry goal- and reward-related information, the summation of these signals at the level of the NAc likely combines information about rewards and risks to direct decision-making. Previous work suggests the medial orbitofrontal cortex (MO) incorporates risk information to bias behavior towards more conservative options. This effect is perhaps due to the inhibition of reward-related circuits such as basolateral amygdala (BLA)-NAc pathways. Using pharmacological inactivation of MO and NAc, we found that MO-NAc interactions permit specific risk assessment behaviors in adult rats. Using in vivo electrophysiology, we also found that MO stimulation modulated the timing and probability of BLA-evoked NAc spiking in a time- and frequency-dependent manner. Single-pulse MO stimulation reduced BLA-evoked NAc spike latency, whereas MO train stimulation produced a frequency-dependent effect on BLA-evoked NAc spike probability. MO train stimulation delivered at 10 Hz facilitated BLA-evoked NAc spike probability but diminished the interactions at 40 and 60 Hz. These opposing effects by MO train stimulation may reflect biases in optimal decision-making, where a subject is inclined towards large-reward/high risk or small-reward/low-risk choices based on previous outcomes and overall long-term benefit. Further, we found that adolescents exhibit fewer risk assessment behaviors than adults during elevated plus-maze exploration. In parallel electrophysiology recordings we found

that MO inputs to NAc exert a unique effect in adolescents. Unlike adults, the adolescent MO failed to produce the bimodal effects on BLA-NAc interactions; MO train stimulation diminished BLA-NAc spiking at all tested frequencies. These effects may reflect suboptimal adolescent decision-making. While the matured MO can regulate the strength of reward-related circuits, the immature MO cannot produce the same bimodal effect to guide decision-making appropriately. Collectively, these results indicate that the development of the MO and its afferents may underlie the age-dependent differences seen in decision-making.

Disclosures: M.K. Loh: None. **N.C. Ferrara:** None. **J.M. Torres:** None. **J.A. Rosenkranz:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.06

Topic: H.03. Decision Making

Support: R01-DA032758
R01-MH104494
McDonnell Center for Systems Neuroscience pre-doctoral fellowship

Title: Neuronal origins of choice biases under sequential offers

Authors: *W. SHI[1], S. N. BALLESTA[2], C. PADOA-SCHIOPPA[1];
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Abstract: Shedding light on choice biases observed behaviorally is a long-term goal of neuroeconomics (Glimcher and Rustichini, 2004), but progress on this front has been arguably modest. Here we examined the neural origins of choice biases measured when goods available for choice are offered sequentially. In the experiments, monkeys chose between two juices offered in variable amounts. In each session, two choice tasks were randomly interleaved. In Task 1, offers were presented simultaneously; in Task 2, offers were presented in sequence. Comparing choices across tasks revealed three phenomena: (I) Monkeys were substantially less accurate in Task 2 compared to Task 1. (II) Choices in Task 2 were biased in favor of the second offer (order bias). (III) Choices in Task 2 were biased in favor of the preferred juice (preference bias).
Choosing between goods entails a series of operations. In broad strokes, sensory information and the internal state are integrated to compute the subjective value of individual offers. Offer values are then compared in a decision process. Finally, the decision outcome, possibly held in working memory, guides the construction of some action plan. Critically, choice biases may emerge

during any of these computations. Previous work identified in the orbitofrontal cortex (OFC) different groups of neurons encoding individual offer values, the binary choice outcome and the chosen value. These variables capture both the input and output of the decision process, suggesting that decisions might be formed within OFC (Padoa-Schioppa and Conen, 2017). Recent work showed that choices under sequential offers engage the same cell groups (Shi et al., 2021). If so, suitable analyses of their activity may shed light on the origins of choice biases. Building on this ideas, we examined the activity of 1,204 cells from the OFC of two monkeys. Our analyses revealed that different biases emerged at different computational stages. Specifically, the lower choice accuracy observed in Task 2 offers emerged, at least partly, at the valuation stage (weaker offer value signals). The order bias found in Task 2 did not have neural correlates during valuation, and emerged during the decision. Finally, the preference bias found in Task 2 emerged late in the trial, possibly due to failures in working memory.
In conclusion, offer values integrate multiple dimensions on which goods may vary. Naturally, all of these dimensions influence choices. Adding to this account, our results provide a more articulated picture, in which choices are also affected by biases emerging during the decision or in subsequent computational stages.

Disclosures: W. Shi: None. **S.N. Ballesta:** None. **C. Padoa-Schioppa:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.07

Topic: H.03. Decision Making

Support: OD011132
5T32NS096050-23
MH117103

Title: The medial orbitofrontal cortex controls value-based action through neurotrophin systems and interactions with the ventral hippocampus

Authors: *E. P. WOON[1], A. ELBASHEIR[1], S. L. GOURLEY[2];
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Abstract: Value-based decision making, referring to selecting actions based on the value of prospective outcomes, relies on the medial orbitofrontal cortex (MO), yet molecular mechanisms are unclear. One candidate is the Brain-derived Neurotrophic Factor receptor, tyrosine/tropomyosin receptor kinase B (TrkB). Here, we trained mice to respond for 2 distinct food outcomes. We next reduced the value of one outcome using conditioned taste aversion (CTA), then characterized decision-making strategies. We administered a TrkB antagonist during

outcome value updating (during the CTA procedure) or during a period of value memory retrieval (the choice test). Inhibiting TrkB during the updating, but not retrieval, of memories regarding food value impaired the ability of mice to preferentially respond for high-value foods. Both overexpression of an inactive isoform of TrkB, *Trkb*.t1, and knockdown of the active receptor, TrkB.FL, selectively in the MO had the same consequences, particularly when outcomes were unobservable and had to be envisioned. What inputs to the MO might be needed to update the value of outcomes? We determined that vHC-to-MO projections are essential for updating the value of outcomes to guide future action selection. In particular, preliminary evidence suggest that vHC-to-MO projections facilitate the processing of aversive homeostatic state information to influence later decisions. Preliminary evidence also indicates that vHC-to-MO connections require TrkB activity on MO neurons to update outcome value for future action selection. In summary, these patterns suggest neurotrophin activity in the MO is necessary for value-based action, as are inputs from the vHC to the MO. Future experiments will determine whether value updating influences dendritic spines on excitatory neurons in the MO that specifically receive input from the vHC.

Disclosures: E.P. Woon: None. **A. Elbasheir:** None. **S.L. Gourley:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.08

Topic: H.03. Decision Making

Support: NIH training grant T32-GM008602
NIH R01DA044297
NIH R01MH117103
Children's Healthcare of Atlanta
NIH P51OD011132

Title: Mice prone to habits exhibit compulsive-like behavior: Possible involvement of orbitofrontal melanocortin 4 receptor

Authors: *S. T. YOUNT[1], A. G. ALLEN[2], S. L. GOURLEY[2];
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Abstract: Adjusting familiar actions based on changes in the surrounding environment is part of everyday life. Goal-directed behavior (i.e., making a choice based on changes in outcome expectation) is antithetical to habitual behavior, which is instead triggered by cues and not outcome predictability. Habitual behavior is adaptive under many circumstances, but it could be

antecedent to compulsions - intrusive thoughts that drive persistent and potentially maladaptive behavior. Hyperactivity of the orbitofrontal cortex (OFC) is a hallmark characteristic of patients with obsessive compulsive disorder, and melanocortin 4 receptor (MC4R) signaling may be involved. We hypothesized that mice with a natural propensity for habitual behavior would exhibit compulsive-like behavior. We selectively bred mice that fail to update instrumental behaviors when expected rewards are not delivered and instead defer to familiar behavioral sequences. Their offspring developed the same response biases and compulsive-like and risk-taking behavior. Compulsive-like behavior was corrected by fluoxetine and correlated with instrumental response strategies. We next hypothesized that compulsive-like behavior may be attributable MC4R systems in the OFC. Repeated, but not acute, chemogenetic stimulation of MC4R+ glutamatergic OFC neurons triggered compulsive-like grooming. A prominent theory of addiction and compulsive disorders is that they involve a loss of control over habits. Understanding whether action strategies correlate with compulsions, and whether melanocortin signaling alters cortico-striatal connectivity, will advance future efforts to mitigate harmful habitual behavior.

Disclosures: S.T. Yount: None. **A.G. Allen:** None. **S.L. Gourley:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.09

Topic: H.03. Decision Making

Support: NINDS Grant R37NS21135 (RTK)
R21MH109851 (MH, RTK, IS)
DM was the recipient of the (i) Fulbright and (ii) Yad Hanadiv (Rothschild) Postdoctoral Fellowships.

Title: To share or not to share? iEEG evidence for state-dependent inequity encoding in the OFC

Authors: *D. MARCIANO[1], B. R. STAVELAND[1], J. LIN[2], I. SAEZ[3], M. HSU[1], R. T. KNIGHT[1];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]Dept. of Neurol., Univ. of California, Irvine, Irvine, CA; [3]Univ. of California Davis, Davis, CA

Abstract: A wealth of functional neuroimaging and lesion evidence has pointed to the involvement of the human orbitofrontal cortex (OFC) in social valuation and decision-making. However, due to inherent limitations of spatiotemporal resolution of these methods, mechanistic understanding of OFC's role in underlying processes have remained elusive. Here we sought to shed light on these questions by conducting intracranial recordings in neurosurgical patients

while they performed a social decision-making task. Nine intracranial patients (808 electrodes, 101 OFC electrodes) played a repeated Dictator Game, a task in which participants unilaterally decide on the allocation of a money between themselves and an anonymous other. On each trial, patients chose between two options: an equitable, constant option ($10 for themselves, and $10 for the other), and a second inequitable option which varied from trial to trial. The Inequity of the varying option came in one of two types: (i) advantageous inequity (AI) where the dictator received more (e.g., $15 and $8 respectively), and (ii) disadvantageous inequity (DI) where the dictator received less (e.g., $12 and $22). Consistent with suggestions from neuroimaging studies, we found extensive reward-related representations (e.g., own payoff, maximal payoff) in High Frequency Activity (HFA; 70-150 Hz) in the OFC, as well as whether the varying option contained advantageous or disadvantageous inequity with a significant effect in 43% of OFC electrodes (permutation test: $p < 0.05$). In addition, owing to improvements of temporal resolution and signal to noise (SNR), we found strong evidence for state-dependent encoding of these reward-related representations. Specifically, encoding depended on the nature of inequity of the varying option—i.e., whether the inequity of the varying option was advantageous or disadvantageous. For example, 34% of OFC electrodes encoded maximal value only in DI trials, but not in AI trials, and 43% of electrodes encoded the other's payoff only in advantageous trials, but not in disadvantageous trials. Together, these results support the idea that OFC high-frequency activity encodes relevant social decision-making computations in a context-sensitive way. Our findings are consistent with notions that OFC encodes the current abstract space of a task (a global "task space"), which in turns modulates the neural representation of valuation computations (Niv et al., 2014; Zhou et al., 2019; Shuck et al., 2016); and extend the previous literature to social contexts.

Disclosures: D. Marciano: None. **B.R. Staveland:** None. **J. Lin:** None. **I. Saez:** None. **M. Hsu:** None. **R.T. Knight:** None.

Poster

P792. Orbitofrontal Cortex: Circuitry and Neurochemistry

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P792.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R15DA046375

Title: Mu-opioid receptors in the medial orbital cortex contribute to cross-hemispheric regulation of reward-guided behavior

Authors: *S. R. WHITE, C. B. DARDEN, K. CHAVEZ LOPEZ, M. LAUBACH; American Univ., Washington, DC

Abstract: The medial orbital cortex (mOFC) is a key frontal region for processing of reward information that is enriched with mu-opioid receptors. Reversible inactivation of mOFC reduces incentive contrast effects and palatability measures (Parent et al., 2015). Neuronal activity in mOFC is modulated when rats initiate consummatory licking (Horst and Laubach, 2013) and encodes the value of liquid sucrose rewards (Amarante et al., 2017). Few studies have examined the roles of mOFC mu-opioid receptors in reward-guided behavior. We tested rats in an incentive contrast licking task and a value-guided decision making task (Swanson et al., 2021) following infusion of muscimol and the selective mu-opioid receptor agonist DAMGO. In the licking task, rats were trained to lick on a contact-sensitive spout to receive access to liquid sucrose, which alternated between high (16%) and low (4%) values over 30sec periods. With training, rats learned to persistently lick for the higher value fluid and suppress licking for the lower value fluid. Bilateral infusion of DAMGO (1μg/μL), targeting mOFC, slowed licking rates and increased the number of licking bouts and reduced their duration, without increasing overall consumption or altering relative reward value. By comparison, infusion into the lateral ventricle, targeting the entire forebrain, increased consumption and had mixed effects on microstructural measures of licking. In the decision making task, rats nosepoked to produce visual stimuli, with the brightness of the stimuli associated with their reward value. Bilateral infusions of both muscimol (0.1 ug/ul) and DAMGO (1 ug/ul) led to dramatic disruptions of performance, suggestive of impaired procedural memory. We further examined neural correlates of these effects in the licking task by implanting a drug cannula in one hemisphere and a microwire array in the other. Unilateral DAMGO infusions increased delta (<4 Hz) power, disrupted phase locking at the lick frequency, and enhanced phase locking at higher frequencies. Together these data suggest that mu-opioid stimulation in the mOFC disregulates cross-hemispheric rhythmicity which may lead to the changes in reward-guided behaviors.

Disclosures: S.R. White: None. **C.B. Darden:** None. **K. Chavez Lopez:** None. **M. Laubach:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R15DA046375.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.01

Topic: H.04. Executive Functions

Support: NIH NIGMS- GM113109
start up funds to Dr. Plakke from KSU

Title: Adolescent female valproic acid rats are impaired on the extra-dimensional shift of attention

Authors: C. KING, T. DAVISON, Z. E. MCKINNELL, B. CHALLANS, *B. PLAKKE; Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: The valproic acid (VPA) model of Autism Spectrum Disorder (ASD) is a widely accepted model of early environmental exposure to sodium valproate to induce ASD-like symptoms in offspring. It has both face and construct validity. Previous research has found that VPA rats are impaired at attentional set-shifting in adulthood (McKinnell et al., 2021). The current research examined if the same cognitive deficits can be observed earlier in development during adolescence. Pregnant dams were injected on gestational day 12 with VPA (600mg/kg) or saline. It was hypothesized that VPA rats would perform worse on the extradimensional shift (ED) phase of the attentional set-shifting task. The study used N=32 Long-Evans rats (9 male controls, 7 VPA males, 9 control females, 7 VPA females). All of the rats were trained to complete the set-shifting task on postnatal day 35-38. Male and female data were analyzed separately based on the lab's previous findings that VPA females and males perform differently on the set-shifting task (McKinnell, et al., 2021). Researchers running behavioral tests were blind to treatment conditions. Preliminary data analysis demonstrates that female VPA rats performed significantly worse on the ED phase of the task compared to controls ($p<0.01$). These results support the hypothesis that executive function deficits in females are occurring within ASD at an earlier age compared to males. Ongoing research is measuring the volumes of the medial prefrontal cortices (mPFC) of these rats, which is the critical structure for ED performance. These data support the hypothesis that the VPA model is capturing some of the same cognitive deficits present in humans with ASD, which allows for a greater understanding of the underlying neurobiology of the disorder.

Disclosures: C. King: None. **T. Davison:** None. **Z.E. McKinnell:** None. **B. Challans:** None. **B. Plakke:** None.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.02

Topic: H.04. Executive Functions

Title: The role of D_4 receptors in cognitive flexibility and ADHD

Authors: *Z. MCKINNELL[1], T. MAZE[1], C. KING[1], T. DAVIDSON[1], B. PLAKKE[2]; [1]Psychological Sci., [2]Psychological Sciences, Behavioral Neurosci., Kansas State Univ., Manhattan, KS

Abstract: Impairments in cognitive flexibility are found in a wide range of psychiatric disorders, such as schizophrenia, Parkinson's disease, and attention-deficit hyperactivity disorder (ADHD). These disorders are all linked to altered dopamine (DA) network processing (Klanker et al., 2013), therefore; understanding the role of DA in models of cognitive flexibility and attention is crucial for determining disease progression and designing effective treatments at each disease stage. Although, medications are available for treating ADHD it is still important to find the most effective and efficient treatment options for individuals diagnosed with ADHD or other similar disorders. Using a D_4 antagonist, SHR and Long-Evans were treated with the drug over a 14-day period to simulate real-life treatment and was run through the marble-burying task, open-field Test, and the attentional set-shifting task, which is the rodent equivalent of the Wisconsin Card Sorting Task (Birrell et al., 2000). The marble task and the open-field test were used to test multiple side effects of the drug and will be used to determine whether the SHRs exhibit the appropriate symptomology for ADHD. Previous studies have identified that the variability in ADHD symptoms in SHRs is extremely high, which could lead to effects being masked and unimpaired rats being assumed to be impaired (Ha et al., 2020). Behavioral profiling is currently underway, but preliminary analyses have indicated that control SHRs were impaired across multiple phases of the attentional set-shifting task, while SHRs in the treatment group performed marginally better across the task. Long-Evans in the treatment group performed worse than control Long-Evans, which could be due to the drug treatment having a negative effect on our control strain due to their lack of need. This is not surprising given that individuals who take unprescribed ADHD medication perform worse on cognitive tasks. These results will add to the literature about whether more specific DA drugs will work more efficiently than non-selective treatments, which have many unwanted side effects for treated individuals.

Disclosures: Z. McKinnell: None. **T. Maze:** None. **C. King:** None. **T. Davidson:** None. **B. Plakke:** None.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.03

Topic: H.04. Executive Functions

Support: University of Memphis bridge funding and a faculty research grant (H.J.K.S.)
University of Cincinnati Graduate School Dissertation Completion Award (S.L.R.)
NIH R01ES032270 (C.V.V. and M.T.W.)

Title: Phasic Dopamine Release Measurements in Two Different Rat Models of Attention-Deficit/Hyperactivity Disorder: Spontaneously Hypertensive Versus Lphn3 Knockout Rats

Authors: *P. NALAN[1], R. L. PACE[1], C. B. HICKS[1], S. L. REGAN[2,3], M. T. WILLIAMS[2], C. V. VORHEES[3], H. J. SABLE[1], D. B. LESTER[1];
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Abstract: Attention-Deficit/Hyperactivity Disorder (ADHD) is associated with a dysfunction in dopamine transmission and is often treated with drugs that increase extracellular dopamine levels. The present study examined dopamine release in the nucleus accumbens core (NAc) of two rat models of ADHD. Spontaneously Hypertensive Rats (SHRs) are an accepted phenotypic ADHD model that exhibit impulsivity/hyperactivity and inattention. *Lphn3*$^{-/-}$ (knockout, KO) rats are a genetic model that also exhibit impulsivity/hyperactivity, but to a lesser degree than the SHRs. The *Lphn3*KO rats were created based on data from a genome-wide, multigenerational study that linked variants in the *Lphn3*(*Adgrl3*) gene [OMIM 616417] to ADHD. Using *in vivo* fixed potential amperometry, we quantified stimulation-evoked phasic dopamine release in the NAc of the *Lphn3* KO and *Lphn3*$^{+/+}$ (wildtype, WT) rats as well as SHRs and their control strain, Wistar-Kyoto (WKY) rats, before and after administration of nomifensine (10 mg/kg, ip), a dopamine transporter (DAT) blocker. Both SHRs and *Lphn3* KO rats displayed decreased baseline dopamine release compared to their respective controls, with no difference between sexes. However, the dopaminergic response to DAT inhibition differed. Sex effects were observed in SHR rats, with male SHR rats displaying a decreased dopaminergic response to nomifensine compared to WKY males and female SHR rats responding similarly to WKY females. No differences were observed between male and female *Lphn3* KO rats; both sexes displayed an increased dopaminergic response to nomifensine relative to WTs. These findings indicate differences in DAT functioning between SHR and *Lphn3* KO rats and suggest that DAT inhibitors, such as those used to treat ADHD, may produce different therapeutic outcomes.

Disclosures: P. Nalan: None. **R.L. Pace:** None. **C.B. Hicks:** None. **S.L. Regan:** None. **M.T. Williams:** None. **C.V. Vorhees:** None. **H.J. Sable:** None. **D.B. Lester:** None.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.04

Topic: H.04. Executive Functions

Support: NIMH (MH72672)
Veterans Administration (BX003512)

Title: Ventral hippocampal input to the infralimbic cortex is necessary for the effects of extinction on set shifting after chronic stress

Authors: *D. PAREDES[1], S. WAY[1], D. A. MORILAK[2];
[1]Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX; [2]Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Psychiatric disorders such as post traumatic stress disorder and major depressive disorder are characterized by deficits in cognitive flexibility. We have established extinction learning in rats as a behavioral intervention that models the effects of exposure therapy on cognition. Extinction reverses chronic stress-induced deficits in cognitive flexibility on the attentional set-shifting test (AST), a medial prefrontal cortically-mediated executive process. Extinction requires the activity of pyramidal neurons in the infralimbic cortex. The ventral hippocampus plays a key role in regulating infralimbic activity during extinction learning, and plasticity in the ventral hippocampus is necessary for extinction memory consolidation. Thus, in these experiments we set out to investigate the role of ventral hippocampal input to the infralimbic cortex (IL) for the therapeutic effects of extinction after chronic stress. Further, we investigated whether chronic stress induces deficits in the novel object recognition test (NOR), and whether the effects of stress were reversed by extinction learning. Male and female rats received viral injections of inhibitory or control designer receptor exclusively activated by designer drugs (DREADDs) into the ventral hippocampus. Subsequently, rats were fear conditioned or received tone control treatment and underwent chronic unpredictable stress (CUS). Prior to extinction treatment, rats received a bilateral microinjection of CNO (200umol) into the IL. 24 hours after, rats were tested on the NOR test and AST. Our results show that inhibiting ventral hippocampal input to the IL during extinction blocks the therapeutic effects of extinction (n=4-6/group, p=0.0045, males and females). Preliminary data show that stress may induce a deficit on NOR, ongoing experiments will determine whether extinction reverses impairments on NOR (n=4-6/group, p=0.4). Supported by research grants from the NIMH (MH72672), the William and Ella Owens Foundation, and the Veterans Administration (BX003512).

Disclosures: D. Paredes: None. **S. Way:** None. **D.A. Morilak:** A. Employment/Salary (full or part-time):; South Texas Veteran's Health Care System, San Antonio TX. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); H. Lundbeck, Copenhagen, Denmark.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.05

Topic: H.04. Executive Functions

Support: Chan-Zuckerburg Biohub Award

IOCDF Breakthrough Award
Robert Wood Johnson Harold Amos Medical Faculty Development Program
R01MH105461

Title: Ketamine increases activity of a fronto-striatal projection that regulates compulsive behavior in SAPAP3 knockout mice

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Abstract: Obsessive-Compulsive Disorder (OCD), characterized by intrusive thoughts (obsessions) and repetitive behaviors (compulsions), is associated with dysfunction in fronto-striatal circuits. There are currently no fast-acting pharmacological treatments for OCD. However, recent clinical studies demonstrated that an intravenous infusion of ketamine rapidly reduces OCD symptoms. To probe mechanisms underlying ketamine's therapeutic effect on OCD-like behaviors, we used the SAPAP3 knockout (KO) mouse model of compulsive grooming. Here we recapitulate the fast-acting therapeutic effect of ketamine on compulsive behavior, and show that ketamine increases activity of dorsomedial prefrontal neurons projecting to the dorsomedial striatum in KO mice. Optogenetically mimicking this increase in fronto-striatal activity reduced compulsive grooming behavior in KO mice. Conversely, inhibiting this circuit in wild-type mice increased grooming. Finally, we demonstrate that ketamine blocks the exacerbation of grooming in KO mice caused by optogenetically inhibiting fronto-striatal activity. These studies demonstrate that ketamine increases activity in a fronto-striatal circuit that causally controls compulsive grooming behavior, suggesting this circuit may be important for ketamine's therapeutic effects in OCD.

Disclosures: G.L. Davis: None. **A.R. Minerva:** None. **A. Lario:** None. **L.D. Simmler:** None. **C. Rodriguez:** A. Employment/Salary (full or part-time):; stipend from APA Publishing for her role as Deputy Editor at The American Journal of Psychiatry. 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.; research grant support from Biohaven Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); served as a consultant for Epiodyne. **L.A. Gunaydin:** None.

Poster

P802. Executive Function and Inhibitory Control in Rodents

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P802.06

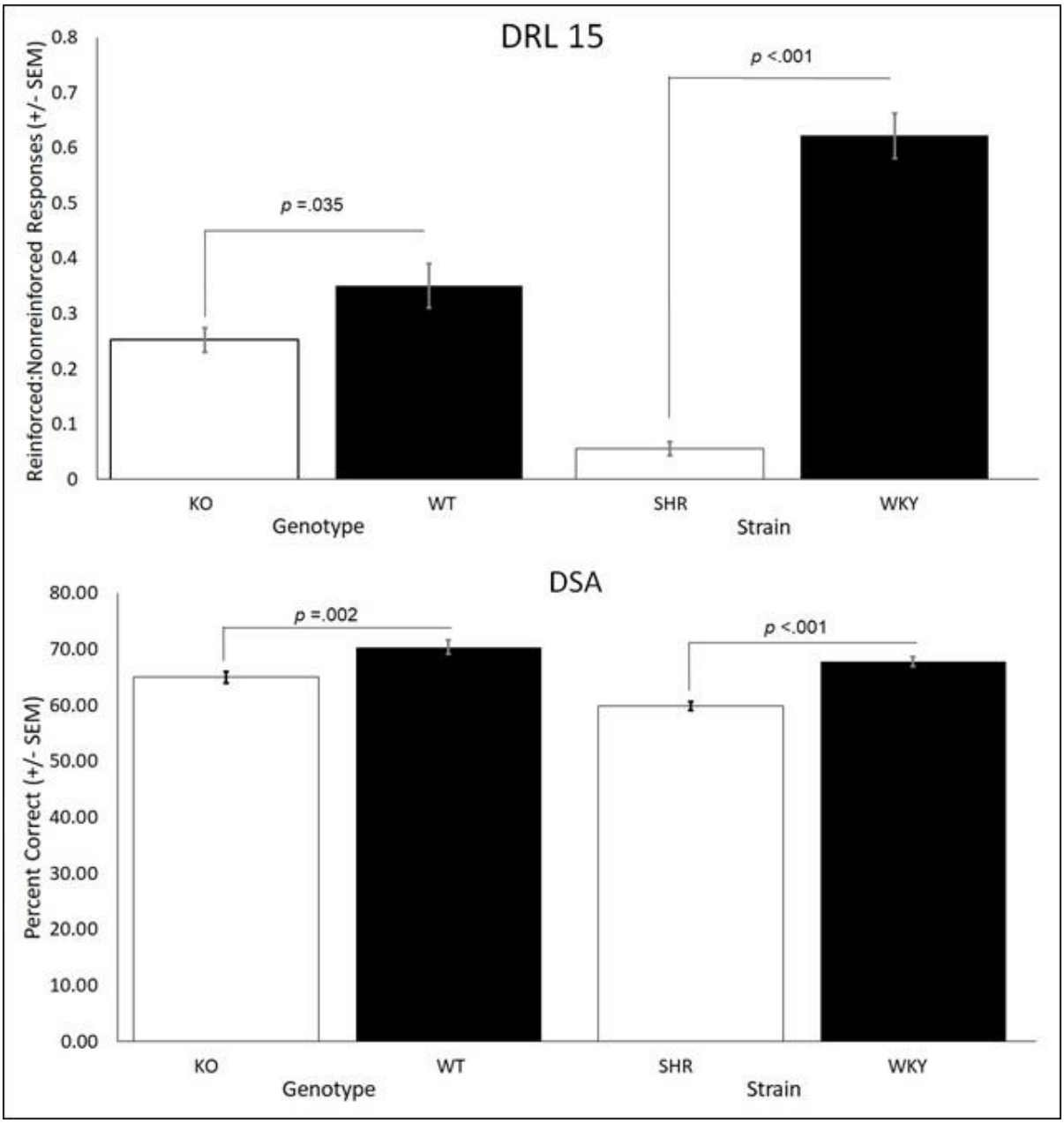
Topic: H.04. Executive Functions

Support: University of Memphis bridge funding (H.J.K.S)
University of Memphis faculty research grant (H.J.K.S.)
University of Cincinnati Graduate School Dissertation Completion Award (S.L.R.)
NIH R01ES032270 (C.V.V. and M.T.W.)

Title: Impulsive action and working memory impairments in two different rat models of Attention-Deficit/Hyperactivity Disorder: Spontaneously Hypertensive versus Lphn3 knockout rats

Authors: *H. J. K. SABLE[1], D. B. LESTER[1], J. L. POTTER[1], H. G. NOLEN[1], D. M. CRUTHIRD[1], L. M. ESTES[1], A. D. JOHNSON[1], S. L. REGAN[2,3], M. T. WILLIAMS[2,3], C. V. VORHEES[2,3];
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Abstract: Attention-Deficit/Hyperactivity Disorder (ADHD) is characterized by two primary presentations - hyperactivity/impulsivity and inattention - which can occur individually or together, thereby resulting in three unique ADHD sub-types: hyperactive/impulsive, inattentive, or combined. Recent data from a genome-wide, multigenerational sample linked variants in the *Lphn3* (*Adgrl3*) gene [OMIM 616417] to ADHD. While, previous research has demonstrated *Lphn3*$^{-/-}$ (i.e., knockout, KO) rats are hyperactive compared with *Lphn3*$^{+/+}$ (i.e., wildtype, WT) rats, this project was done to examine the impact of *Lphn3* deletion in rats on operant measures of impulsive action and working memory. Impulsive action was measured via a differential reinforcement of low rates of responding (DRL) task in which rats were required to wait 15 s (i.e., DRL 15) between lever presses to earn a food reinforcer. Working memory was assessed using a delayed spatial alternation (DSA) task that required rats to alternate lever presses from one trial to the next to earn the food reinforcer. Random delays of 0, 5, 10, or 20 s were imposed between trials during DSA. “Positive control” measures on the same tasks were collected in Spontaneously Hypertensive Rats (SHRs), which are an accepted animal model of ADHD. Compared with WT controls, the *Lphn3* KO rats exhibited deficits on both the DRL and DSA tasks, indicative of deficits in impulsive action and working memory, respectively. These deficits were less severe than those observed in SHRs, who were *profoundly* impaired on both tasks compared with their control strain, Wistar-Kyoto rats (WKY). When combined with previous research, both the L*phn3* KO rats and the SHRs encompass the impulsive/hyperactive presentation of ADHD. While previous research has also shown the SHR rat to have attentional impairments (thus representing the combined ADHD subtype), additional research is necessary to assess whether the L*phn3* KO rats are also inattentive, or whether they represent only the hyperactive/impulsive sub-type.



Disclosures: H.J.K. Sable: None. **D.B. Lester:** None. **J.L. Potter:** None. **H.G. Nolen:** None. **D.M. Cruthird:** None. **L.M. Estes:** None. **A.D. Johnson:** None. **S.L. Regan:** None. **M.T. Williams:** None. **C.V. Vorhees:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.01

Topic: H.05. Working Memory

Support: R01EY016407

Title: Unveiling the abstract format of mnemonic representations

Authors: *Y. KWAK, C. CURTIS;
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Abstract: The precise contents of working memory (WM) can be decoded from the patterns of neural activity in human visual cortex (Harrison and Tong, 2009; Serences et al., 2009), leading to the idea that the same encoding mechanisms are used for perception and WM maintenance (Serences, 2016). However, results are inconsistent as to whether sensory representations are good predictors of memory representations (Albers et al. 2013; Rademaker et al., 2019), and distracting sensory inputs sometimes have negligible effects on WM (Bettencourt and Xu, 2016; Hallenbeck et al., under revision; Lorenc et al., 2018). One reasonable hypothesis of WM representation that accounts for these inconsistencies is that mnemonic codes are perhaps abstractions of low level stimulus features. Yet the format of these putative abstract representations has thus far remained impenetrable. Here, we demonstrate that different types of visual stimuli can be flexibly re-coded into the same WM format when that representation is useful for memory-guided behavior. Specifically, we found that the pattern of activity in visual and parietal cortex during WM for dot motion and for gratings, two very different retinal inputs, are interchangeable when participants were later tested on the global direction of the motion or the orientation of the grating. To reveal the representational format of WM, we then used simulations and models that reconstructed WM representations within topographic populations. We found evidence that the behaviorally relevant feature of each type of stimulus (motion direction and orientation) was extracted and re-coded into a shared mnemonic representation that takes the form of an abstract line-like pattern within spatial topographic maps. These findings suggest that mnemonic representations can take on formats that are efficient summaries of perceptual representations with more proximal links to the behavioral affordances contingent on the memory.

Disclosures: Y. Kwak: None. **C. Curtis:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.02

Topic: H.05. Working Memory

Support: ONR Grant N00014-17-1-2041
Swartz Foundation
NIH Grant R01MH062349
Simons Collaboration on the Global Brain program grant 543057SPI

Title: Bifurcation in hierarchy space as a mechanism for distributed working memory in a large-scale model of the macaque neocortex

Authors: J. JIANG[1], U. PEREIRA OBILINOVIC[2], X.-J. WANG[3];
[1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]Ctr. for Neural Sci., [3]New York Univ., New York, NY

Abstract: Numerous electrophysiological and imaging studies in non-human primates and humans demonstrate the distributed nature of working memory. Working memory-related sustained activity is rarely observed in the sensory areas but robustly displayed in the prefrontal, parietal, and inferotemporal association cortices. What are the organizing principles that determine the pattern of distributed working memory activity across the cortex? To address this question, we use a modeling framework that integrates a generative model for the mesoscopic connectivity of the macaque neocortex (Song et al, 2014) with biophysically realistic neuronal dynamics. First, we use the diffusion map method on the generated connectivity data to estimate the hierarchical position of each cortical region. Using this hierarchy, we define a macroscopic gradient for the strength of excitation across the cortical mantle, consistent with experimental observations. Then, we use the resulting connectivity and gradient of excitation to construct a large-scale dynamical model of the macaque neocortex. Our model naturally displays multiple distributed working memory-related persistent activity patterns, consistent with experimental observations. Strikingly, only higher hierarchical regions display persistent activity while lower areas present background activity. There is a sudden transition in firing rate activity along the hierarchy, determining which brain region will present working memory-related persistent activity. This phenomenon corresponds to a bifurcation in the hierarchy space. The critical hierarchical position when this transition occurs corresponds to the bifurcation point. We find that areas around the bifurcation point present slow fluctuations or switching between background and persistent activity. Thus, the time scale of cortical areas around the bifurcation point increases dramatically to seconds. As reported in association areas, the time scale for cortical areas after the bifurcation could also reach seconds, while areas lower in the hierarchy have much shorter intrinsic time scales. Together, our work suggests that working memory states are organized around a bifurcation in cortical hierarchy. Signatures of this bifurcation are predicted to be present in cortical time scales.

Disclosures: J. Jiang: None. **U. Pereira Obilinovic:** None. **X. Wang:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.03

Topic: H.05. Working Memory

Support: R00DA042934 (NIDA)
R00DA042934-03S1 (NIA)
T34GM127154 (NIGMS)
Summer Medical Research Fellowship Program (Rowan University SOM)

Title: Transient inactivation of the nucleus reuniens, but not dorsal hippocampus, impairs performance on a delayed nonmatch to position task

Authors: S. D. DUNN[1], E. J. CIACCIARELLI[1], T. GOHAR[1,2], P. C. KUMPF[1], P. D. KUMPF[1], T. J. SLOAND[1,3], M. NIEDRINGHAUS[1], ***E. A. WEST**[1,3];
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Abstract: Working memory is the ability to hold information online to guide behavior and is critical for navigating the environment. The rat medial prefrontal cortex (mPFC), the dorsal hippocampus (dHPC) and their functional connection, are required for working memory using a delayed nonmatch to sample task in a radial arm maze. Since there is no direct projection between the mPFC and dHPC, the thalamic nucleus reuniens (RE) is a likely intermediary between these structures due to its anatomical connections and role in spatial working memory. Here, we aimed to determine if RE and dHPC are required for performance on a delayed nonmatch to position (DNMTP) task. This task consists of a 1) sample phase (one lever extended, e.g., right) 2) time delay (2-32s) and 3) a choice phase, initiated by nosepoke, where the opposite lever (i.e., nonmatch) from the sample phase must be pressed (e.g., left) in order to receive a reward. Failure to press the sample lever, nosepoke, or press the choice lever, resulted in a trial omission. Fischer F344 rats (males and females) were trained on the DNMTP task. Once criterion (80% correct, 50 trials, 2s delay) was met, rats underwent stereotaxic surgery for bilateral implantation of infusion guide cannulae into RE (n=6) or dHPC (n=7). Rats were then trained in the task with 4 distinct delays (last session: 4, 8, 16, 32 s). Rats then received bilateral intracerebral infusions of the GABAA agonist muscimol (0.5 mM) or equal volume saline infusion into the RE (0.1 ul) or dHPC (0.3 ul) with the order of drug treatment counterbalanced across rats. We found that pharmacological inactivation of the RE led to overall decreased performance on the DNMTP task as indicated by a decrease in percent correct across all delays. Specifically, we found a significant main effect of drug treatment (muscimol vs saline) across delays ($F(1,5) = 14.84$, $p = 0.01$) but no effect of delay ($F(1.631, 8.155) = 1.51$, $p=0.27$). Pharmacological inactivation of the dHPC, however, did not lead to overall decreased performance on the DNMTP. We found no significant effect of drug treatment ($F(1.276, 25.52) = 0.293$, $p=0.65$) but an effect of delay ($F(3,20) = 14.33$, $p=0.0001$). In addition, even when we increased the muscimol concentration (1mM and 5mM) we found no effect of dHPC inactivation on DNMTP performance. Thus, transient inactivation of the RE, but not dHPC, impaired overall performance DNMTP in a delay independent manner. Ongoing studies are examining how

transient inactivation of the mPFC affects performance on the DNMTP. Finally, we will use inhibitory chemogenetic manipulation to determine if the mPFC-RE and the RE-mPFC circuits are required for behavioral performance.

Disclosures: S.D. Dunn: None. **E.J. Ciacciarelli:** None. **T. Gohar:** None. **P.C. Kumpf:** None. **P.D. Kumpf:** None. **T.J. Sloand:** None. **M. Niedringhaus:** None. **E.A. West:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.04

Topic: H.05. Working Memory

Support: NSF 2050833

Title: Simultaneous visual and motor selection in working memory

Authors: *R. WEESE[1], E. F. ESTER[2];
[1]Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Psychology, Univ. of Nevada, Reno, NV

Abstract: Working memory enables adaptive behavior by forming a temporal bridge between prior sensations and anticipated future actions, and a large body of research suggests that working memory storage and action planning are tightly coupled. Under some circumstances, the selection of information stored in working memory and the selection of linked motor plans occurs in parallel, enabling both fast and accurate behavior. This arrangement is advantageous in controlled settings where mappings between the contents of memory and specific actions remain constant, but potentially disadvantageous in more complex settings where memory representations and motor plans must be constantly updated to match changing environmental circumstances. Thus, in this study we ask whether memory representations and associated motor plans are invariably accessed simultaneously, even when there is no incentive to do so. We recorded EEG while human volunteers remembered the orientation of two Gabor-like stimuli over a short delay, then adjusted a probe stimulus to match one of the remembered items. At the end of each trial, participants adjusted a probe Gabor to match one of the remembered stimuli. A valid retrospective cue presented one second prior to the onset of the response period informed volunteers which Gabor stimulus would be probed. Following earlier work (van Ede et al., Nature Neuroscience, 2019) we linked different stimuli to specific affordances (left vs. right hand button presses) and independently manipulated the location, color, and orientation of the to-be-remembered stimuli. This allowed us to independently assess the time-course of visual and action selection through an analysis of lateralized occipitoparietal alpha-band (8-13 Hz) and frontocentral beta-power (15-35 Hz), respectively. We observed simultaneous changes in these signals beginning approximately 300 ms after the onset of the retrospective cue, consistent with a

parallel selection of visual and motor information. This occurred even though a one-second gap separated cue and probe onset, minimizing the necessity of parallel selection. These findings suggest that mechanisms responsible for visual and motor selection are tightly coupled, even when task circumstances do not demand it.

Disclosures: R. Weese: None. **E.F. Ester:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.05

Topic: H.05. Working Memory

Support: AG063775
Philanthropic support

Title: Frequency tradeoff in neural variability predicts working memory success

Authors: *F. R. BAUMGARDT, V. VISWANATHAN, O. F. TWEEN, R. M. REINHART; Dept. of Psychological & Brain Sci., Boston Univ., Boston, MA

Abstract: Recurring fluctuations in neural activity have been proposed as a mechanistic foundation for behavior. Modulation in neural variability across trials is a general cortical event, appearing in numerous brain regions and elicited by different stimuli and tasks in animals and humans. Here, we examine the effects of working memory load on ongoing neural variability in humans. While signal enhancement has been thoroughly investigated in human and animal models, changes in neural variability as a result of working memory load has received substantially less attention. Further, human working memory neuroscience has largely focused on averaged delay-period activity rather than the neural dynamics within the delay. Using human electroencephalography and a delayed-match-to-sample task, we show that as more complex real-world objects are loaded into memory, an opposing pattern of behaviorally relevant cross-trial neural variability is observed between low- and high-frequency oscillations. Variability in delta (2-4 Hz) and theta (4-8 Hz) bands was positively correlated with upcoming memory accuracy within the delay period and across set sizes. However, as set size increased, the spatial distribution of memory-related theta variability shifted across the head from posterior to anterior, and generally decreased over time within the delay, with the largest set size inducing more temporally sustained variability predictive of later behavior. In contrast, cross-trial variability of high-frequency oscillations in beta (13-30 Hz) and gamma (30-60 Hz) bands were negatively correlated with memory accuracy. High-frequency memory variability was absent early within the delay and for low set sizes but steadily increased with time and memory load, particularly over central-parietal regions. Further, correlations between neural variability and total power

were scarce, and total power did not predict memory performance for any frequency band, set size, or time-point within the delay, suggesting that memory-related neural variability was not merely a correlate of signal power. The findings suggest that working memory behavioral success is mediated by a tradeoff between early low-frequency oscillatory enhancement and later high-frequency oscillatory diminution in trial-by-trial neural variability, underscoring the importance of spontaneous neural variability for improved working memory.

Disclosures: F.R. Baumgardt: None. **V. Viswanathan:** None. **O.F. Tween:** None. **R.M. Reinhart:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.06

Topic: H.05. Working Memory

Support: P20GM103430, Rhode Island IDeA Network of Biomedical Research Excellence (RI-INBRE) Early Career Development Award

Title: The P300 event related potential as a predictor of phonological working memory skills in school-aged children

Authors: *V. HARWOOD, A. BARON;
Univ. of Rhode Island Interdisciplinary Neurosci. Program, Kingston, RI

Abstract: Phonological processing skills play a significant role in the development of word reading ability (Nelson et al., 2012), yet the neural substrates of phonological processing in children remains unknown. One main component of phonological processing is phonological working memory (PWM) (Wagner et al., 1993). Nonword repetition tasks are the gold-standard clinical tool used in measuring PWM. Further, the P300 Event Related Potential (ERP) has been cited as an index of PWM (Bonala & Jensen, 2012). The current study investigates the prognostic power of the P300 component in predicting behavioral measures of PWM skills in a cohort of monolingual English and bilingual Spanish-English early school-aged children. Forty-two typically-developing 1st and 2nd grade children between the ages of 6.1 and 9.1 years of age (M: 7.3 years, 15 Female, 27 Male) participated in a battery of standardized language and literacy assessments as well as an electrophysiological task. Thirty-three students were monolingual and 9 students were identified as bilingual through behavioral assessments and parent interviews. The ERP task consisted of a classic auditory oddball paradigm task in which one block contained English contrasts (/ta/ standard, /pa/ deviant) and another block contained Spanish contrasts (/t̪a/ standard, /d̪a/ deviant). The deviant trials occurred 20% of the time. Children were instructed to listen to the sounds and press a button when they heard the deviant

tone. The P300 component was identified in a cluster of 10 parietal electrodes. Latency and amplitude data between 250-500 ms post stimulus onset were extracted for both the averaged standard and deviant trials. Difference waves (deviant - standard) were created for all participants. The latency difference within the P300 ERP component recorded in response to auditory English speech contrasts significantly predicted English nonword repetition scores on the Comprehensive Test of Phonological Processing, 2nd Edition (CTOPP-2) for both monolingual and Spanish-English bilingual children (R^2 = .18), F(1, 37) = 7.81, p = .01. The P300 ERP component recorded in response to auditory Spanish speech contrasts did not significantly predict English nonword repetition scores for either group of children (R^2 = .01), F(1, 37) = .51, p =.48. The P300 component recorded in response to English phonemes is a strong predictor of behavioral English PWM skills as measured by a nonword repetition task in young school-aged monolingual and bilingual children. The P300 component as a measure of PWM has potential diagnostic and therapeutic relevance to clinicians working with school-aged children.

Disclosures: V. Harwood: None. **A. Baron:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.07

Topic: H.05. Working Memory

Support: NINDS R37NS21135
Brain Initiative U19 U19NS107609
Research Council of Norway, project number 240389
RITMO, project number 262762
NINDS K99NS115918

Title: Frontotemporal dynamics of verbal working memory revealed by iEEG

Authors: *A. LLORENS[1], L. BELLIER[1], E. L. JOHNSON[1], J. IVANOVIC[3], P. G. LARSSON[3], T. ENDESTAD[4], A.-K. SOLBAKK[4], R. T. KNIGHT[2];
[1]Helen Wills Neurosci. Inst., [2]Univ. of California, Berkeley, Berkeley, CA; [3]Dept. of Neurosurg., Oslo Univ. Hosp., Oslo, Norway; [4]Univ. of Oslo, Oslo, Norway

Abstract: Keeping in mind the items chosen from a menu while waiting to order requires verbal Working Memory (vWM). Cognitively, vWM consists of three sequential phases: encoding (storing information in memory), maintenance (keeping information in memory), and probe (recalling information from memory). vWM studies typically focus on one of these three phases, involvement of one brain region, or modulation of one specific neural frequency band. Detailed

analysis of the entire vWM process is typically lacking. We recorded intracranial electroencephalography (iEEG) from fronto-temporal regions of eleven patients with intractable epilepsy. We analyzed the time course of four frequency bands: theta, alpha, beta, and high frequency activity (HFA; [70-150Hz]), as vWM unfolded. The task consisted of a list of 5 letters presented in succession (displayed for 500-ms with a 500-ms interstimulus interval), followed by a 4-s maintenance phase, and then a 2-s probe asking participants to answer whether a given letter was on the list. Participants performed the task with an average of 81% correct answers. The percentage of correct negative answers was significantly higher than the positive ones (87% vs. 75%, $p<.05$), consistent with previous literature suggesting that it is easier to correctly reject a lure not presented in the trial than to confirm the presence of an item. Electrophysiological analyses showed significant frequency band effects throughout vWM phases ($p<.001$), which were distinct across four clusters of electrodes defined along two anatomical axes: left vs. right hemisphere and frontal vs. temporal lobes. Cluster-based permutation analysis revealed significant power increases in the low frequencies (theta and alpha) in temporal regions bilaterally during the encoding and the maintenance phases ($p<.05$). In addition, a power correlation analysis identified a left alpha-beta frontotemporal network which tracked memory load with the presentation of each letter during encoding. Presentation of the probe elicited HFA in frontal regions bilaterally, but no correlation was found between hemispheres. Collectively, these results suggest an electrophysiological signature of the language network due to the verbal aspect of the task, followed by a dissociable signature of activity in the frontal lobes when decision making is required. These findings provide evidence of complex coordination of multiple frequency bands in bilateral frontotemporal regions in support of vWM.

Disclosures: A. Llorens: None. **L. Bellier:** None. **E.L. Johnson:** None. **J. Ivanovic:** None. **P.G. Larsson:** None. **T. Endestad:** None. **A. Solbakk:** None. **R.T. Knight:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.08

Topic: H.05. Working Memory

Title: Eeg signals represent updated memory representations in visual working memory

Authors: *Y. SHIN[1], S. L. SHEREMATA[2];
[1]Ctr. for Complex Systems and Brain Sci., [2]Psychology, Florida Atlantic Univ., Boca Raton, FL

Abstract: Visual short-term memory (VSTM) creates stable internal representations over time. Working memory, in addition, allows us to manipulate or update these representations. Visual representations stored in VSTM have been successfully reconstructed from measurements of neural activity. However, it is unclear whether the updated state of a remembered item is

reflected in these neural representations. The present study addressed whether representations reflect updated representations using electroencephalography (EEG) by asking participants to update the orientation of a remembered Gabor. In the task, participants were instructed to mentally rotate a grating based on a color cue that indicated whether participants should rotate the remembered item 1) clockwise, 2) counter-clockwise, or 3) not at all. Then the orientation of the remembered item was decoded from alpha-band oscillations (8-12Hz), associated with visual attention, and sustained EEG potentials (<6Hz), associated with visual short-term memory. We found that reconstructions from sustained EEG (< 6 Hz) reflected updated representations as well as initial sensory representations with higher reconstruction probability at the target orientation while alpha-band frequency (8-12Hz) computed broad scope of reconstructions of target orientations. These results suggest that the neural signal carries not only the sensory information but also manipulated memory representations. Moreover, precision of mnemonic representations was higher in sustained EEG potentials compared to alpha-band oscillations. These results demonstrate that visual short-term memory representations can be updated to reflect behaviorally-relevant information in the absence of perceptual input.

Disclosures: Y. Shin: None. **S.L. Sheremata:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.09

Topic: H.05. Working Memory

Support: Sloan Research Fellowship
US Army Research Office W911NF-19-2-0026

Title: Spatial recoding of oriented gratings maintained in visual working memory revealed by predictive modeling of fMRI activation patterns

Authors: *K. VU-CHEUNG, T. C. SPRAGUE;
UC Santa Barbara, Santa Barbara, CA

Abstract: When participants are remembering the precise orientation of a grating, activation patterns measured from primary visual cortex can be used to decode which orientation value is held in working memory (WM; Serences et al, 2009; Harrison & Tong, 2009), and the quality of decoding is related to behavioral performance (Ester et al, 2013; Albers et al, 2013). Results from these studies support the sensory recruitment model of WM by which neurons responsible for representing perceived visual features additionally are recruited for representing those features during WM (Serences, 2016). However, for a feature like orientation, it may equivalently be the case that participants strategically recode the remembered grating orientation

using a spatial code (e.g., attending to a location on the screen and/or imagining a line). In such a coding scheme, the pattern of activation would still depend on the remembered feature, so linear classifiers or inverted encoding models trained on grating orientations would still perform accurately. Here, we tested whether participants use a spatial code during an orientation WM task by building a forward model of orientation WM based on spatial voxel receptive field (vRF) models (e.g., Mackey et al, 2017). If it is the case that visual information in working memory is being converted into spatial information, we would expect to see voxels with vRF positions aligned parallel with the remembered orientation to exhibit higher activation than voxels aligned to the orthogonal orientation. Here, participants performed a visual WM task where they maintained the precise orientation of a grating, then reported that orientation after a 12 second delay. Additionally, we identified each voxel's spatial selectivity during a vRF mapping session. On each trial, we sorted voxels based on their vRF preference into 'parallel' and 'orthogonal' groups based on their spatial position relative to the remembered orientation and compared their mean activation levels during the delay. This analysis, which involves no free parameters, tests the explicit forward model that an oriented grating is recoded into a specific spatial format. In primary visual cortex, the parallel voxels exhibited greater delay-period activation than orthogonal voxels, consistent with the spatial recoding hypothesis. These results are consistent with previous reports that participants store information in the code most meaningful for upcoming behavior (Lee et al, 2013; Henderson et al, pp2021). Additionally, this approach whereby we generate model-based predictions of activation patterns during a WM task can be used to further probe neural coding schemes during cognitive behaviors.

Disclosures: K. Vu-Cheung: None. **T.C. Sprague:** None.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.10

Topic: H.05. Working Memory

Support: UWA-RTP scholarship for the first Author

Title: Nabilone Impairs Spatial and Verbal Working Memory in Healthy Subjects

Authors: *F. KASSIM[1], S. TOD[1], M. ALBRECHT[2], S. HOOD[1], J. W. LEE[1], J. RODGER[1], M. MARTIN-IVERSON[1];
[1]Univ. of Western Australia, Western Australia, Australia; [2]Curtin Univ., Perth, Australia

Abstract: Several studies have reported that memory-related impairments and psychotic-like experiences are adverse effects of cannabis uses. However, data on the acute and chronic cognitive impacts of cannabis use are not consistent. The present study aimed to investigate the

effect of acute cannabinoid receptor activation on verbal (VWM) and spatial (SWM) working memory by administering oral doses of synthetic cannabinoid agonist, nabilone (1-2 mg, PO). The study also aimed to investigate the effect of nabilone on schizotypy (psychosis-proneness) scores and resultant associations with WM, VWM and SWM.
Methods: Healthy participants completed spatial span and digit span across different delay conditions for WM (n = 28) and psychological testing for schizotypy measures (n = 25) in each of two days after receiving nabilone (1-2 mg, PO) or placebo in a randomized, double-blind, counterbalanced, crossover manner.
Results: SWM performance exhibited significant decreases with delay ($p = 0.0005$), but VWM performance increased with delay indicating likely rehearsal or attentional processes inconsistent with a good measure of WM. Nabilone impaired SWM ($p = 0.00016$) in a delay-dependent manner and VWM ($p = 0.03$) independent of delay, indicating that it was influencing VWM performance via non-memory processes, likely attention. Nabilone did not significantly change schizotypy scores. Schizotypy scores were negatively correlated with WM ($\rho = -0.43$, $p = 0.028$) and VWM ($\rho = -0.35$, $p = 0.07$) but not with SWM ($\rho = -0.24$, $p = 0.23$) across averaged delay condition.
Conclusion: low doses of synthetic cannabinoid (nabilone) impaired SWM and VWM performance indicating that exogenous activation of the cannabinoid system impaired cognitive performance. Furthermore, the relationship between WM impairment and schizotypy in healthy individuals suggests that whatever produces this delay-independent effect (likely attention) on WM performance deficit may be a cognitive dysfunction in people with a schizophrenia spectrum disorder.

Disclosures: F. Kassim: A. Employment/Salary (full or part-time):; University of Western Australia. **S. Tod:** None. **M. Albrecht:** A. Employment/Salary (full or part-time):; Curtin University. **S. Hood:** A. Employment/Salary (full or part-time):; University of Western Australia. **J.W. Lee:** A. Employment/Salary (full or part-time):; University of Western Australia. **J. Rodger:** A. Employment/Salary (full or part-time):; University of Western Australia. **M. Martin-Iverson:** A. Employment/Salary (full or part-time):; University of Western Australia.

Poster

P822. Distributed Mechanisms in Working Memory

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P822.11

Topic: H.03. Decision Making

Support: Simons Collaboration on the Global Brain 543005
HHMI
Wellcome Trust
Pew Scholars Program

NIH Grant NS112312
NIH Grant NS113110
R01EB028171

Title: Multi-regional dynamics of short-term memory explored with brain-wide Neuropixels recordings and multiple statistical models

Authors: *Y. LIU[1], S. CHEN[2], L. D. LIU[3,2], B. KANG[1], H. HOU[3,2], N. LI[3,2], K. SVOBODA[2], S. DRUCKMANN[1];
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Abstract: Persistent neural activity encoding short-term memory (STM) is observed in many brain regions, but how brain regions interact to maintain STM is unclear.
We used up to five Neuropixels probes to simultaneously record the activity of hundreds of neurons across more than a dozen brain regions during a memory-guided delayed response task in the mouse. Anterior lateral motor cortex (ALM; part of M2) is an essential hub for the planning and execution of memory-guided directional licking. We targeted regions that form multi-regional networks with ALM, including nuclei in thalamus, basal ganglia, midbrain, pons, medulla, cerebellum and others (see Susu Chen et al SFN 2021). Here, we compare neural coding across brain areas and employ statistical models to understand brain region interactions under STM.
We decoded stimulus and choice information in individual brain regions. Although coding was distributed across brain regions, individual areas exhibited distinct dynamics. Stimulus information emerged first in midbrain, followed by thalamus and ALM. ALM exhibited a distinct ramp-up of choice information during the memory epoch.
We assessed coordination of choice-related information by projecting population activity into a subspace related to choice (coding dimension, CD). Simultaneously recorded brain areas were highly correlated on a single-trial level.
We compared three statistical methods to infer communication strength between brain regions [1]. Communication subspace analysis and canonical correlation analysis perform dimensionality reduction on the full population activity to maximize variance explained or correlation across populations respectively. The influence model examines the influence of one brain region on another along specific dimensions (e.g. CD) [1]. All models found stronger communication between ALM and connected compared to unconnected brain regions. Inferred communication strengths predicted the impact of optogenetic perturbations on downstream brain regions. However, only the influence model predicted changes in selectivity induced by perturbations. Communication strengths were dynamically regulated across behavioral epochs. These results reveal spatial and temporal interactions between brain regions and their relations to neural coding of task information.
[1] Kang & Druckmann, Curr. Opin. in Neurobiol. 2020

Disclosures: Y. Liu: None. **S. Chen:** None. **L.D. Liu:** None. **B. Kang:** None. **H. Hou:** None. **N. Li:** None. **K. Svoboda:** None. **S. Druckmann:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01 MH113071
Adelson Medical Research Foundation

Title: Organization of memory ensembles across brain regions

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Abstract: Although memory formation, consolidation and retrieval engage neuronal ensembles in multiple brain regions, little is known concerning the principles and mechanisms that coordinate the dynamic interactions between these ensembles. For example, in Conditioned Taste Aversion (CTA), studies have shown that silencing memory-encoding neurons in either the basolateral Amygdala (BLA) or insular cortex (IC), impairs memory storage and retrieval. Since these regions are inter-connected, we hypothesized that IC and BLA coordinate CTA memory ensembles through direct networks that connect neurons in these two regions. To test this hypothesis, we used CREB expression (diverts memory allocation to targeted neurons) and Rabies monosynaptic retrograde tracing to detect whether neurons in memory ensembles in one region project to other memory ensembles in the connected regions. Using c-fos immunostaining as a marker for neuronal activation, we find that connected memory networks in the BLA and the IC are 2.5 times more likely to take part in CTA memory, compared to a) chance levels, b) to local memory neurons, and c) to control groups. We also find that these interregional memory networks are specific to task-relevant brain regions, and they are not present in regions unrelated to the learning task used (e.g., CTA versus auditory conditioning). By expressing CREB in both the BLA and the IC, we drove an increase in both connectivity between CREB expressing cells and, importantly, memory performance: CREB-expressing neurons in the IC were 2 times more likely to connect to CREB-expressing neurons in the BLA, compared to the control group. Dual CREB activation (in the BLA and IC) also resulted in an increase in performance in the CTA task. Modeling studies of our results revealed the surprising finding that a retrograde mechanism coordinates memory ensembles in BLA and IC, suggesting that beyond anterograde mechanisms that establish memory ensembles across brain regions, retrograde mechanisms have a role in fine tuning this complex and dynamic process.

Disclosures: A. Lavi: None. **M. Sehgal:** None. **F. Sisan:** None. **A. Okabe:** None. **D. Ter-Mkrychyan:** None. **A.J. Silva:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.02

Topic: H.08. Learning and Memory

Support: NIH Grant U19NS107609

Title: Learning-to-learn emerges from learning to efficiently reuse neural representations

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Abstract: It is far easier to learn new information when its content is familiar. Our brain engages internal representations of relevant prior knowledge, known as *schemas*, to improve how we interpret and learn new information. In tandem, schema-based learning prospectively refines the schema's effectiveness in promoting future learning. *Learning-to-learn* is a result of this reciprocity, whereby learning a series of problems with shared task structure is accompanied by a progressive speedup in learning. We trained a Recurrent Neural Network (RNN) model on a series of visuomotor mapping problems to identify the neural correlates of a schema, and study the circuit-level mechanisms of this reciprocal improvement. In each problem, the RNN learns to map two high-dimensional inputs (images) onto two outputs (saccades). The trial structure and outputs are shared by problems, but the inputs change across them.
We found that RNNs robustly learn-to-learn: the number of trials to learn a problem decreases exponentially with the number of previously learned problems, consistent with the exponential speedup in association task learning that we observed in macaque monkeys. A subspace decomposition of the RNN's neural activity patterns revealed that the shared task variables, i.e. the trial structure, decisions and choices, are represented consistently across problems by shared low-dimensional neural manifolds. These manifolds are generalized from prior learning, reused to solve new problems, and to control the model's output responses. Importantly, their reuse improves the RNN's learning efficiency. Thus, the shared manifolds form a neural substrate of a visuomotor mapping schema.
While large neural activity changes are evident during learning, analysis showed that they mostly stem from reusing neural representations. This curtails the formation of new representations, which limits the requisite connection weight changes (CWCs) and accelerates learning. We introduce a measure of the role of CWCs in learning — the *weight-driven vector field change* — which showed that CWCs are mainly recruited to preserve the representational structure during learning. Finally, we identified a novel mechanism for learning-to-learn: The CWCs in early

problems prospectively improve the RNN's ability to preserve its representational structure while learning later problems. This further limits the requisite CWCs in later problems and accelerates learning. The accumulation of these prospective CWCs over previously learned problems elicits learning-to-learn. These findings link changes in neural activity and network structure to offer a mechanistic explanation for learning-to-learn.

Disclosures: V. Goudar: None. **B. Peysakhovich:** None. **E.A. Buffalo:** None. **D.J. Freedman:** None. **X. Wang:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.03

Topic: H.08. Learning and Memory

Support: Fi2 GM133534
ZIA DA000587

Title: Anterior cingulate neurons signal valueless associative information during sensory preconditioning

Authors: *E. HART[1], M. P. H. GARDNER[1], G. SCHOENBAUM[2];
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Abstract: Studies of anterior cingulate cortex (ACC) function have primarily employed tasks where subjects learned about or used information that involved items of clear biological significance, or where subjects were required to respond. What these had in common was the same general structure in which one event followed another. It is therefore possible that ACC neurons could simply encode associations between events, irrespective of value. Sensory preconditioning provides an opportunity to test this. Rats (n = 8) were implanted with microelectrodes for recording single units (n = 1,307 total) in ACC and trained in a sensory preconditioning task. During the first phase, preconditioning, rats were presented with two separate pairs of neutral auditory cues. To reveal latent learning of the cue-cue pairings, rats then received reward conditioning during which one of the previously neutral cues was paired with food. Following this, rats were given a probe test during which they were presented with never-reward-paired cues. Rats showed higher responding to the cue that predicted the food-paired cue in this probe test, indicating that the neutral cue pair information was learned during preconditioning. During preconditioning, single ACC neurons fired differently to different sensory cues, and population-level analyses revealed that the neurons developed firing patterns that classified cues into pairs. Further, the development of this encoding of the cue pairs by ACC

neurons during preconditioning predicted appropriate behavioral responding to the preconditioned cues during the probe test. These findings demonstrate that ACC serves a broader role in learning than previously thought, contributing to the formation of valueless associative structure when items of biological significance are not involved.

Disclosures: E. Hart: None. **M.P.H. Gardner:** None. **G. Schoenbaum:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.04

Topic: H.08. Learning and Memory

Support: Simons Foundation (542955)
McKnight Endowment Fund for Neuroscience
National Science Foundation Graduate Research Fellowship

Title: Task structure governs activity in the monkey hippocampus

Authors: *J. W. RUECKEMANN[1,3], Y. BROWNING[2,1], A. MALLORY[1], B. KIM[1], C. I. O'LEARY[1], A. L. FAIRHALL[1], E. A. BUFFALO[1,3];
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Abstract: Hippocampal neurons have been most often studied in rodents running mazes, resulting in a rich history characterizing their activity as spatial correlates. However, these neurons have been recently shown to respond in a variety of tasks without an explicit spatial component, suggesting that hippocampal activity patterns more generally reflect the progression of experience through salient task events.
To examine hippocampal activity in primates, we analyzed data from three rhesus macaques performing a delayed alternation task in a virtual maze - a task chosen to facilitate comparison to the rat literature. Neurons were recorded from the hippocampus in three monkeys using chronically-implanted hyperdrives housing 124 independently movable electrodes. We characterized spatial specificity using two reference frames: avatar position in the maze and location of the monkey's gaze in the virtual world. Approximately 30% of neurons exhibit reliable activity relative to avatar position in the maze as determined through a GLM framework and an information theoretic approach. A majority of these neurons also demonstrate reliable activity with respect to the animal's gaze location. Direct comparison of these variables demonstrates that avatar location better predicts spiking; however, high correlation of these variables prevents a strong interpretation of these results. Notably, many neurons exhibit fields that cover wide expanses of a maze arm, standing in contrast to the punctate place fields

observed in rats and raising the possibility that precise location is not the best predictor of the neuronal activity. In addition, ostensibly spatial activity is not evenly distributed through the maze; key event locations, including the start, choice, and goal locations, are overrepresented in the neural population, suggesting that the wide spatial fields might instead reflect parcellation of the maze into distinct task phases. This interpretation is further evidenced by distinct neural populations that are selectively active during the reward and delay periods. We also examined neural activity in distinct environments by changing the visual appearance of the maze. While some neurons "remap" their responses, the majority of neurons maintain their task phase-specific responses irrespective of the visual cues defining the environment. The stability of activity across distinct contexts suggests that the hippocampus is operating on a schematized representation of this well-learned task. In combination with the parcellation of the representation by salient events, these data indicate that representing task structure is foundational to activity in the hippocampus.

Disclosures: J.W. Rueckemann: None. **Y. Browning:** None. **A. Mallory:** None. **B. Kim:** None. **C.I. O'Leary:** None. **A.L. Fairhall:** None. **E.A. Buffalo:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.05

Topic: H.08. Learning and Memory

Support: R01AG013622
MH113071
Adelson Foundation
Swiss National Science Foundation (postdoc mobility)

Title: A role for the Locus Coeruleus dorsal CA1 dopaminergic projection in the modulation of contextual memory ensembles and contextual memory linking

Authors: A. CHOWDHURY[1], ***A. LUCHETTI**[1], G. FERNANDES[1], D. ALMEIDA FILHO[1], A. TZILIVAKI[2], G. KASTELLAKIS[2], E. RAMIREZ[1], M. TRAN[1], P. POIRAZI[2], A. J. SILVA[3]; [1]UCLA, Los Angeles, CA; [2]IMBB-FORTH, Heraklion, Crete, Greece; [3]Dept Neurobiol, UCLA Med. Ctr., Los Angeles, CA

Abstract: Our brain not only encodes memories of individual events, it can also link memories encoded close in time, such that the recall of one memory triggers the recall of the other. For example, our lab showed that when two contextual memories are encoded close in time (e.g., 5 hours apart) the recall of one triggers the recall of the other (i.e., the two memories are linked). Importantly, our lab also showed that the overlap between the two contextual memory ensembles

in dorsal CA1 (dCA1) is key for contextual memory linking. A number of factors such as salience, reward and novelty are known to affect memory encoding, but it is unclear whether and how these factors affect memory linking. Neuromodulatory projections are thought to have a role in sending information about novelty to dCA1, including dopaminergic/noradrenergic projections from Locus Coeruleus (LC) and serotonergic projections from the Raphe Nucleus (RN). Although both the LC and RN are activated by contextual exploration, only inhibition of LC to CA1-projecting neurons disrupted contextual memory linking. Importantly, this same manipulation did not disrupt contextual learning and memory. Furthermore, pharmacological manipulations demonstrated that this effect was mediated by dopamine and not by noradrenaline. Studies with head mounted fluorescent microscopes (UCLA miniscopes) in dCA1 showed that the manipulations that disrupted contextual memory linking also impaired the overlap between dCA1 contextual memory ensembles observed during contextual memory linking. Additionally, we found that the inhibition of LC to CA1-projecting neurons also affected the synchrony of firing between subpopulations of dCA1 neurons activated by both contextual memories, as well as their activity patterns within cell assemblies. These results report the first modulatory pathway specific to memory linking, they support a key role for the overlap between dCA1 memory ensembles in contextual memory linking, and they reveal novel circuit properties that may underlie memory linking.

Disclosures: A. Chowdhury: None. **A. Luchetti:** None. **G. Fernandes:** None. **D. Almeida Filho:** None. **A. Tzilivaki:** None. **G. Kastellakis:** None. **E. Ramirez:** None. **M. Tran:** None. **P. Poirazi:** None. **A.J. Silva:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.06

Topic: H.08. Learning and Memory

Support: Human Frontier Science Program
Children Tumor Foundation
R01 MH084315

Title: Role of type I interferon signaling and microglia in the abnormal L-LTP and object place recognition deficits of male mice with a mutation of the Tuberous Sclerosis 2 gene

Authors: *M. F. LOPEZ-ARANDA[1], G. M. BOXX[2], M. PHAN[1], I. HERRERA[1], S. TALOMA[1], R. MANDANAS[1], K. BACH[1], C. THADANI[1], E. LUGO[1], O. LU[1], K. DINGLE[1], R. BUI[1], S. LIU[3], N. LI[3], Y. ZHOU[3], G. CHENG[2], A. J. SILVA[1];
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Res., [2]Microbiology, Immunol. and Mol. Genet., Univ. of California Los Angeles, Los Angeles, CA; [3]Physiol. and Pathophysiology, Sch. of Basic Med. Sci., Qingdao Univ., Qingdao, China

Abstract: Tuberous Sclerosis Complex (TSC) is a genetic disorder associated with high rates of intellectual disability and autism. A mouse model of TSC, with a heterozygous null mutation of the Tsc2 gene ($Tsc2^{+/-}$), is associated with deficits in hippocampal dependent tasks as well as abnormal late long term potentiation (L-LTP) in the hippocampal CA1 region. Previous studies in our laboratory that we reproduced here, indicate that these deficits can be reversed in adults by rapamycin, an inhibitor of mammalian Target of Rapamycin (mTOR) function. Here, we show that microglia and type I interferon signaling (IFN1) have a key role in the object place recognition (OPR; a hippocampal dependent task) deficits and abnormal L-LTP of $Tsc2^{+/-}$ male mice. Unexpectedly, we demonstrate that male, but not female, $Tsc2^{+/-}$ mice showed OPR deficits. Importantly, these deficits can be rescued by depletion of microglia as well as by a manipulation of a signaling pathway known to modulate microglia function (interferon-alpha/beta receptor alpha chain KO). In addition to rescuing the OPR deficits, depletion of microglia also rescued the abnormal L-LTP of the $Tsc2^{+/-}$ mice. Altogether, our results suggest that abnormal IFN1 signaling in microglia cause the abnormal L-LTP and OPR deficits of male $Tsc2^{+/-}$ mice.

Disclosures: M.F. Lopez-Aranda: None. **G.M. Boxx:** None. **M. Phan:** None. **I. Herrera:** None. **S. Taloma:** None. **R. Mandanas:** None. **K. Bach:** None. **C. Thadani:** None. **E. Lugo:** None. **O. Lu:** None. **K. Dingle:** None. **R. Bui:** None. **S. Liu:** None. **N. Li:** None. **Y. Zhou:** None. **G. Cheng:** None. **A.J. Silva:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.07

Topic: H.08. Learning and Memory

Support: NIH Grant MH084315
NIH Grant MH113071
PEW Fellowship to DAF

Title: Impaired stability of hippocampal dorsal CA1 neuronal activity in mouse models of Noonan Syndrome is related to Memory Linking deficits

Authors: *D. ALMEIDA-FILHO, Y. CAI, A. MACALINO, A. J. SILVA;
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Abstract: Interactions between Individual memories are key for adaptive behavior. For example, contextual memories encoded close in time (e.g., 5 hours apart) are linked, such that the recall of one memory increases the likelihood of retrieving the other, a phenomenon referred to as memory linking. The 'Allocate-to-Link' hypothesis of memory linking states that learning triggers a transient increase in intrinsic excitability within the ensemble encoding the first memory such that these cells are more likely to encode a new event experienced hours later. When one of the two memories is recalled this overlap between the memory ensembles drives the recall of the other linked memory. In support of this hypothesis, we have shown that the activity-dependent expression of the cAMP response element-binding protein (CREB) modulates the excitability and the co-allocation of neuronal ensembles for the encoding of distinct memories, thus driving the overlap between these ensembles at the heart of memory linking. Here, we report the results of studies of memory linking in mouse models of Noonan Syndrome (NS), an autosomal dominant disorder that results in the basal overexpression of the phosphorylated extracellular signal-related kinase (pERK), an upstream regulator of the transcription factor CREB. For these experiments, we used NS mice models with a knock-in mutation in *Ptpn11* (*Ptpn11*$^{D61G/+}$) as well as mice with viral overexpression of a NS-associated allele (*PTPN11*^{D61G}) in adult mouse dorsal hippocampus. Although individual contextual memories were normally encoded under the conditions tested, contextual memory linking studied with a 5-hour interval was impaired in these mouse models of NS. Studies with head-mounted miniature microscopes in freely behaving mice showed that, unlike their wild type littermates or animals infused with a control virus, NS mice showed impairments in the stability of individual neuronal activity and of coactivity patterns of dCA1 neurons measured when the mice visited two contexts 5-hours apart. These findings suggest that the stability of neuronal activity dynamics during contextual memory encoding is important for linking distinct contextual memories experienced close in time, and that these dynamic processes are impaired in mouse models of Noonan syndrome.

Disclosures: D. Almeida-filho: None. **Y. Cai:** None. **A. Macalino:** None. **A.J. Silva:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.08

Topic: H.08. Learning and Memory

Support: CONACYT 273553
ANR:ANR-15-CE17-0013

Title: Noradrenergic and glutamatergic transmission during exploratory behavior within the hippocampus and amygdala of a juvenile model of diet induced cognitive impairment.

Authors: *P. SALCEDO-TELLO[1], A. HERNANDEZ-MATIAS[2], D. OSORIO-GÓMEZ[2], F. BERMUDEZ-RATTONI[2], G. PACHECO-LOPEZ[1], G. FERREIRA[3], K. R. GUZMAN-RAMOS[1];
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Abstract: According to the World Health Organization obesity has tripled since 1975. Not only adults have been affected with this obesity epidemic; in 2019, 38 million children and adolescents were overweight or obese. In addition to its association with chronic diseases like hypertension and diabetes, obesity affects neurocognitive features including learning and memory. To assess the effects of juvenile obesity on hippocampal function, we used a model of diet-induced obesity in male Wistar rats. Rats were exposed after weaning to a regular diet (control group, 2.9Kcal/g) or a high-fat diet (HFD group, 4.7Kcal/g) for 3 months thus covering adolescence. HFD induced increased basal glucose blood levels, diminished glucose tolerance and insulin insensitivity. Once completed the diet exposure time, rats were implanted with microdialysis guide cannulae in the ventral hippocampus (VH) and basolateral amygdala (BLA). While rats from both groups were submitted to an exploration task, 4 minutes samples were collected to assess glutamate, noradrenaline and dopamine release from both structures. We found that in the control group, hippocampal norepinephrine release increased during exploration compared to HFD group. Conversely, glutamate release increased within the hippocampus of HFD group compared to control group. Regarding amygdalar activity, we found that during exploration, control animals increased glutamate release, but HFD group levels remain unchanged. During exploration noradrenergic neurotransmission augmented in HFD rats but not in control rats. Dopamine did not show a difference between groups in hippocampus or amygdala. Our results suggest that consumption of HFD during adolescence interfere with normal neurotransmission, besides altering metabolic parameters. This deregulation seems to be differential between structures as seen in hippocampus and amygdala indicating a modulatory role regarding the specific functions on memory trace formation.

Disclosures: P. Salcedo-Tello: None. **A. Hernandez-Matias:** None. **D. Osorio-Gómez:** None. **F. Bermudez-Rattoni:** None. **G. Pacheco-Lopez:** None. **G. Ferreira:** None. **K.R. Guzman-Ramos:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.09

Topic: H.08. Learning and Memory

Support: CONACyT Grant 273553
Agence Nationale de la Recherche Grant ANR-15-CE17-0013

Title: Impact of High-Fat Diet on aversive conditioning and its relation to amygdalar and hippocampal neurotransmission in a juvenile obesity model

Authors: A. HERNANDEZ-MATIAS[1], P. SALCEDO-TELLO[2], F. BERMUDEZ-RATTONI[1], G. PACHECO-LOPEZ[2], G. FERREIRA[3], K. R. GUZMAN-RAMOS[2], ***D. OSORIO-GÓMEZ**[1];
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Abstract: Obesity has become a major health issue around the world; according to the World Health Organization, the prevalence of obesity has alarmingly increased. Excess in energy intake and the subsequent increased adiposity during childhood and adolescence have been observed independently of age, sex, ethnicity or socioeconomic status. Overweight and obesity are related to chronic diseases such as cardiovascular diseases, diabetes, kidney failure and cancer. However, obesity also promotes cognitive deficits including learning and memory. We assessed the impact of high-fat diet intake during adolescence on the formation of an aversive memory and its relation to amygdalar and hippocampal neurotransmission. We used a juvenile obesity model in Male Wistar rats, which were exposed to a regular diet (Chow, 2.9 kcal/g) or a High-Fat diet (HFD, 4.7 kcal/g) for 12 weeks after weaning. According to metabolic parameters, HFD induced an augmentation in weight, adiposity, and basal glucose blood levels. In addition, HFD diminished glucose tolerance and insulin sensitivity. At the final stage of diet, rats were implanted with microdialysis guides aiming at the basolateral amygdala (BLA) and ventral hippocampus (VH). We monitored glutamate, noradrenaline and dopamine within BLA and HV during the acquisition of a Conditioned Odor Aversion (COA) task. Results indicated that HFD animals showed an enhancement of the aversive response in comparison to animals exposed to Chow. This behavioral response is associated to an increase in glutamate and norepinephrine release within the BLA and VH after the administration of a gastric-malaise inductor agent (LiCl). Therefore, HFD exposure during adolescence alters metabolic parameters and enhances emotional-associated memories through the increased neurochemical transmission within the BLA and VH. Thus, adolescence is a vulnerable period where diet-induced obesity impacts on cognition via neurochemical alterations.

Disclosures: A. Hernandez-Matias: None. **P. Salcedo-Tello:** None. **F. Bermudez-Rattoni:** None. **G. Pacheco-Lopez:** None. **G. Ferreira:** None. **K.R. Guzman-Ramos:** None. **D. Osorio-Gómez:** None.

Poster

P852. Learning and Memory: Physiology

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P852.10

Topic: H.08. Learning and Memory

Support: NIH R01 MH067284

Title: Establishing an ex vivo model for aversive associative learning in fruit flies

Authors: *M. ADEL, L. GRIFFITH;
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Abstract: The general rules that govern the *Drosophila melanogaster* associative memory circuit have been characterized by a multitude of *in vivo* studies in the past decade. However, certain challenges associated with experimenting on behaving flies have left outstanding questions regarding the mechanisms that govern the specificity of dopamine release on particular synapses and the precise role of dopamine in encoding associative memory. In mammals, the development of the *ex vivo* LTP model in hippocampal slices revolutionized our understanding of memory formation. Establishing an *ex vivo* model for the fruit fly associative memory circuit would similarly allow for in-depth investigation of the innerworkings of this circuit, while overcoming the limitations of *in vivo* techniques. To accomplish this goal, we developed an *ex vivo* model for aversive associative learning in which pairing activation of the odor pathway with activation of ascending cervical inputs in the dissected fly central nervous system mimics aversive training (pairing an odor with punishment). We show that our model recapitulates the main features of aversive learning: 1) Aversive *ex vivo p*lasticity is specific to the trained odor; 2) This plasticity potentiates the trained odor responses in specific axons of the mushroom body intrinsic neurons and suppresses the trained odor responses in their postsynaptic partners, mushroom body output neurons (enhancement and suppression memory traces). We also demonstrate that pharmacological application of dopamine, or activation of a specific cluster of dopaminergic neurons (PPL1), can replace activation of the punishment pathway, but not the odor pathway, resolving a point of conflict in the literature with a previous *ex vivo* model. We use our preparation to show that one night of sleep deprivation impairs associative memory formation, indicated by failure to form pairing-induced memory traces. This failure was reversed by allowing flies a sleep rebound of 2 hours in early morning. In sum, we established a model that can be reliably utilized to further detail the mechanisms of *Drosophila* associative memory and lays the groundwork for future experiments investigating the dynamics of neural plasticity in this circuit.

Disclosures: M. Adel: None. **L. Griffith:** None.

Poster

P884. Mechanisms of Language Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P884.01

Topic: H.11. Language

Support: CONACYT

Title: A study by diffusion tensor imaging and connectometry analysis, in patients with specific language impairments and patients with autism spectrum disorder

Authors: *F. VILLANUEVA[1], P. DIES-SUAREZ[2], E. BARRAGÁN[3], D. ALVAREZ[3], S. HIDALGO-TOBON[4];
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Abstract: The prevalence of Specific Languages Impairments (SLI) is 9.92%, while for Autism Spectrum Disorders (ASD) it's just over 1%, both disorders may present overlaps in symptoms, which it can complicate its correct diagnosis.Using diffusion weighted images obtained in a 3T scanner, through the diffusion tensor and connectometry [1], 41 patients with a mean age of 7.33 years were studied divided into three groups: controls, TDL and TEA. Through the tensor, 12 Regions of Interest (ROI's) were analyzed: arcuate fasciculus, superior longitudinal, medium longitudinal, inferior longitudinal, inferior fronto occipital and uncinate in both hemispheres, quantifying their parameters of fractional anisotropy (FA), mean diffusivity (MD), axial (AD) and radial (RD), in order to find significant differences in any parameter of any region. Connectometry was used to contrast the results of the diffusion tensor and search new regions where some parameter was correlated with the "diagnosis", which was used as an ordinal variable. The DSI software was used in the ROI study and for the connectometrys.The left arcuate fascicle showed a significant difference between the control and TDL patients, in the MD and AD with p-values of 0.054 and 0.013, while the right superior longitudinal fasciculus the significance appeared in the MD and RD, with p-values of 0.037 and 0.034.The connectometrys yielded various correlations, the most important ones appeared in the quantitative anisotropy (QA), RD and AD in fibers of the corpus callosum, left and right cerebellum and middle cerebellar pedunculum. Connectometry works with many null hypotheses hence the FDR was 0.0616, 0.0004, 0.003 and 0.0092. All these latest regions are not considered in the language organization models, however various studies have correlated them with the lateralization of language (corpus callosum)[2], semantic and phonetic processing, contralateral to the dominant hemisphere of language (cerebellar hemispheres) [3].Although the sample is small and has an impact on the results of descriptive statistics, connectometry, by using robust methods such as permutation and bootstrapping tests, generates more reliable and novel results. This work sought to generate information on SLI and ASD in Spanish-speaking patients, of which not enough information has been generated in the specialized literature.

References

[1] Yeh et al., NeuroImage 125:162-171, 2016. [2] Banich, MIT Press, Cambridge, MA. pp. 427-450, 1995. [3] Arasanz et al., Cortex, 48, 718-724, 2012.

Disclosures: F. Villanueva: None. **P. Dies-Suarez:** None. **E. Barragán:** None. **D. Alvarez:** None. **S. Hidalgo-Tobon:** None.

Poster

P884. Mechanisms of Language Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P884.02

Topic: H.11. Language

Support: Kiwanis Neuroscience Research Foundation
NIMH (1 P50 MH094258)

Title: Left temporal lobe is a convergence region for retrieval of knowledge of unique entities: A neuropsychological study

Authors: *J. KAMINSKI[1], M. BOWREN, Jr[2], K. MANZEL[3], D. TRANEL[4];
[1]Div. of Psychological and Brain Sci., [2]Div. of Neuropsychology and Cognitive Neurosci., [3]Dept. of Neurol., Univ. of Iowa, Iowa City, IA; [4]Dept. of Neurol., Univ. Iowa, Iowa City, IA

Abstract: Following damage to the anterior temporal lobes (ATL), it is not uncommon for patients to experience a new and sometimes profound impairment in the retrieval of proper names. Proper naming is a critical complex function used to identify "semantically-unique" entities; that is, proper nouns are used to denote unique, "one of a kind" entities, such as specific persons or places. Previous research has linked proper naming to the left ATL, whereas retrieval of conceptual knowledge for unique entities ("recognition") has been linked to the right ATL. Here, we studied a sample of 242 (124 female) neurological patients with focal brain lesions to explore the prevalence and neuroanatomical correlates of recognition and naming impairments for two categories of unique entities: famous persons and famous landmarks. Patients were assessed in the chronic epoch ($\geq$3 months post-onset) using the Iowa Famous Faces Test and the Iowa Famous Landmarks Test. Patients were presented with pictures of 145 famous people and 65 famous landmarks and directed to identify the entity by providing the associated proper name. If unable, the patient was subsequently asked to provide sufficient identifying details of the person or landmark to demonstrate recognition. We found that recognition of famous faces and famous landmarks were positively correlated (Pearson's $r(208) = 0.51$, $p < 0.001$). Naming of faces and landmarks were also strongly positively correlated (Pearson's $r(210) = 0.65$, $p < 0.001$). Among
demographic variables, only education had a statistically significant association with the percent recognized and percent named scores for both faces ($F(1, 236) = 23.78$, $p < 0.001$; $F(1, 236) = 14.34$, $p < 0.001$) and landmarks ($F(1, 209) = 57.24$, $p < 0.001$; $F(1, 209) = 17.27$, $p < 0.001$), respectively. Impairments in both recognition and naming for both categories were linked to left-sided ATL lesions. Our results further establish the left ATL as a critical hub in the process of

retrieving a unique lexical entry (proper name) associated with a semantically-unique entity (person, place). The results may reflect recruitment of naming regions during recognition, as many patients provided alternative names (e.g., movie titles, character names, countries, etc.) to demonstrate recognition. Overall, these results are consistent with the existing literature that identifies the temporal lobes as crucial regions for naming and recognition of unique entities.

Disclosures: J. Kaminski: None. **M. Bowren:** None. **K. Manzel:** None. **D. Tranel:** None.

Poster

P884. Mechanisms of Language Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P884.03

Topic: H.11. Language

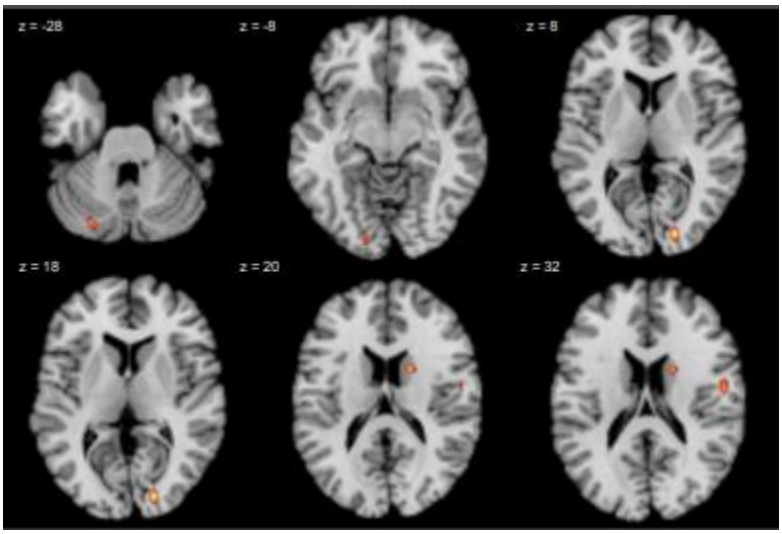
Support: Research reported in this publication/presentation/work was supported in part by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Numbers KL2TR003016/ULTR003015

Title: Functional brain differences due to familial history of dyslexia: a meta-analysis

Authors: J. MORREL[1], *A. J. KRAFNICK[3], T. M. EVANS[2];
[1]Psychology, [2]Sch. of Educ. and Human Develop., Univ. of Virginia, Charlottesville, VA;
[3]Psychology, Dominican Univ., River Forest, IL

Abstract: Introduction: Structural and functional neuroimaging studies have established a network of brain regions associated with developmental dyslexia. While consensus suggests a left lateralized language-oriented network, there are open questions about the heterogeneity of results (Krafnick & Evans, 2019; Ramus et al., 2018). One open area of interest is whether these observed abnormalities are a consequence of reading difficulties or are present even before formal reading instruction has begun (Krafnick et al., 2014; Vandermosten et al., 2016). This study provides an updated synthesis of current functional neuroimaging studies focused on pre- and emerging readers with a family history of dyslexia through meta-analysis. Methods: PubMed and Google Scholar were used to identify neuroimaging studies of pre- and emerging readers with and without a familial risk of dyslexia. Search terms included (dyslexia AND neuroimaging), (pre-readers AND familial risk), (pre-readers AND dyslexia). From these results, studies not using neuroimaging methods, only including literate subjects, and not including a whole brain direct comparison of familial history groups were removed. The remaining fMRI studies were included for meta-analysis using GingerALE 3.0 (Eickhoff et al. 2009, 2012; Turkeltaub et al., 2012). Individual analyses for each direction of family history of dyslexia (FHD+) vs no family history of dyslexia (FHD-) were conducted. Results: Eleven individual fMRI studies were included (n = 499; total foci FHD- > FHD+ contrast = 60; total foci FHD+ >

FHD- contrast = 21). No results were found at an FWE cluster corrected 0.05 threshold. At an uncorrected threshold of p<.001 (min. cluster of 100mm^3), the FHD- > FHD+ contrast showed 6 significant clusters (see Figure). For the FHD+ > FHD- contrast, there was one significant cluster in the right middle frontal gyrus. Discussion: Overall there is some consistency across studies, but only at uncorrected thresholds. This is likely due in part to the diversity in functional tasks and some differences in subject inclusion across studies.



Disclosures: J. Morrel: None. **A.J. Krafnick:** None. **T.M. Evans:** None.

Poster

P884. Mechanisms of Language Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P884.04

Topic: H.11. Language

Support: NIH Grant K08NS110919
NIH Grant P50CA097257
Robert Wood Johnson Foundation grant 74259
The UCSF LoGlio Collective
The Sheri Sobrato Brisson Brain Cancer Fund
NIH grant R00DC013828

Title: Quantifying the causal impact of different sub-regions of tumor lesion on semantic naming ability

Authors: *E. AHN[1], S. DARK[1], O. WIESE[1], C. FINE[1], S. KAKAIZADA[2], J. KAUR[2], S. HERVEY-JUMPER[2], D. BRANG[1];
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Abstract: The ability to accurately name an object and access semantic information is an important component of an individual's social health and vocational outlook. Neuroimaging research has shown involvement of temporal and frontal regions in semantic naming tasks, such as when naming a presented item. However, most methods are unable to show that a brain region is causally involved in a particular behavior, limiting our understanding of the process and lowering the prediction of associated deficits following neurosurgical resection. Voxel-lesion symptom mapping (VLSM) has been widely used to study the causal relationship between brain regions and behavioral impairments at the level of individual voxels by analyzing the continuous behavioral score at each voxel. Applied to patients with a lesion caused by a stroke, VLSM studies have reported a causal relationship between anterior and mid-temporal regions and semantic language functions. However, these studies are biased to identify critical areas around major neuro-vasculature and observed in a restricted clinical population, limiting the generalizability. In contrast, damage by tumors is more evenly distributed throughout the brain and occurs in a broader cross-section of age groups. While tumor-models may provide validation and extension of stroke VLSM findings, few studies have applied VLSM to tumor data, resulting in gaps in what features of a tumor account for variance in VLSM models. Thus, we sought to: (1) Replicate findings from stroke VLSM research to identify regions associated with semantic naming deficits in non-vascular lesions, and (2) Investigate how dimensions that are unique to tumor lesions can further account for variance in VLSM models. Our preliminary findings in *n*=133 individuals validate stroke VLSM research by showing a relationship between semantic naming and damage to the left temporal lobe (maximal at middle and inferior temporal regions). Next, we examined how each histological sub-region of the tumor (i.e., peritumoral edema or core) differentially contribute to semantic naming impairment. While the presence of either edema or core in temporal regions was associated with poor semantic naming, deficits were larger for voxels in the tumor core. Finally, consistent with stroke VLSM research, larger core volume was associated with greater semantic naming impairment regardless of its location, highlighting the importance of covarying this dimension. Our findings suggest that different sub-regions of the tumor differentially impair semantic naming function and that the unequal distribution of tumor cells within the lesion are causally linked to varying levels of language deficit.

Disclosures: E. Ahn: None. **S. Dark:** None. **O. Wiese:** None. **C. Fine:** None. **S. Kakaizada:** None. **J. Kaur:** None. **S. Hervey-Jumper:** None. **D. Brang:** None.

Poster

P884. Mechanisms of Language Disorders

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P884.05

Topic: H.11. Language

Support: NIH Grant P50NS22343
NIH Grant T32DC7361

Title: Language Performance in Children with Early Brain Injuries: Does Location of the Lesion Play a Role as Children Reach School-age?

Authors: *P. T. LAI;
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Abstract: Children with Perinatal Strokes provide a unique opportunity to study the neural basis of communication. These children suffered a brain injury around the perinatal period (i.e. between the last trimester of gestation up to the first month after birth). Lacking the ability to process and effectively convey social information can lead to deficits in maintaining relationships and can lead to academic failure in school. Few studies have investigated the social phenotypes of these children during school-age as the majority of studies occur before the age of 6. The purpose of this study is to explore if children with left (LHI) or right hemisphere injury (RHI) are similar in how they behave/communicate at school age or are they more similar to their typically developing (TD) peers. Our questions: (1) Is the child's injury related to their language performance (Broca's area or Wernicke's area)? (2) Are facial expressions related to injuries especially within the right hemisphere? The children in this project were between the ages of 7-14 and the dataset includes a 7 to 10-minute interview from 8 LHI children, 8 RHI children and 8 TD children. The procedure was to code social interactions using Eudico Linguistic Annotator software to observe various communicative behaviors. From our behavioral dataset, we determined which children are continuing to show the impacts of their injuries and from our imaging dataset, we documented where in the brain the damage occurred. Results showed that LHI children do not talk as much as the other two groups, a result similar to adults who have Broca's aphasia. A second result found that subcortical damage, rather than Broca's area or Wernicke's area resulted in more language deficits in LHI. Lastly, the TD children performed well in producing facial expressions, whereas children with brain injuries had delays/deficits. The two children who struggled the most were in the LHI group. Both had subcortical damage without any damage to Broca's area or Wernicke's area. This result is in line with a prior study where they found similar results regarding subcortical damage leading to language deficits. In that study, it was found that subcortical involvement to either hemisphere may be the most important determiner of failure on certain language task (i.e., syntax). Insights from this study adds unique knowledge to our understanding of communication as well as contribute to better-informed treatment methods in the future.

Disclosures: P.T. Lai: None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.01

Topic: H.12. Aging and Development

Support: Mcknight Brain Research Foundation
NIH/NIA 2RF1AG049722 (SNB)
NIH/NIA K9AG058786 (SAJ)
McNair Scholars Program (AMR)

Title: Development of touchscreen-based mnemonic similarity task to assess cognitive abilities in rodent models

Authors: *A. ROSS[1], S. N. BURKE[2], C. N. LOGAN[3], S. A. JOHNSON[4], J. L. BIZON[5], C. WATSON[1], T. GATTON[1];
[2]Neurosci., [3]Psychology Dept., [4]Dept. of Neurosci., [1]Univ. of Florida, Gainesville, FL;
[5]Neurosci., Univ. of Florida McKnight Brain Inst., Gainesville, FL

Abstract: Age-related cognitive decline and Alzheimer's disease (AD) lead to loss of independence and burden caregivers. To better understand the neurobiological basis of age-related cognitive decline, we need animal models that accurately reflect the deficits observed in humans. Previous work in our lab has demonstrated that aged rats, like older adults, are impaired in their ability to distinguish similar stimuli, referred to as mnemonic discrimination. However, this test was run on a maze and not comparable to how humans are assessed in clinical setting. In moving toward integration of tools for manipulating and recording neural activity with behavioral assessment with higher translational potential, we have utilized a mnemonic similarity task (MST) developed by Lee et al (2015) that can be administered in automated operant touchscreen chambers. In the current study, we trained and tested rats in a single-stimulus presentation version of the touchscreen-based MST. Young (4 months) and adult (21 months) 344 Hybrid x Brown Norway rats of both sexes were first shaped to respond to a visual stimulus presented on the touchscreens by providing liquid reward. Rats were then trained to distinguish pairs of image stimuli (i.e. A vs. B) by associating each stimulus with touching different side of the screen (i.e. A-tap Left, B-tap Right). Rats were then tested by presenting morphed image stimuli that shared 5%, 25%, or 45% overlapping visible features with the previously learned target image stimuli (i.e. A and B). Results show that rats' mnemonic discrimination performance declines on trials when morphed images with greater percent overlapping features are presented. We predict that this task will be particularly sensitive to age-related deficits so that its implementation will be useful in future assessments of cognitive aging and neurodegenerative diseases such as AD.

Disclosures: A. Ross: None. **S.N. Burke:** None. **C.N. Logan:** None. **S.A. Johnson:** None. **J.L. Bizon:** None. **C. Watson:** None. **T. Gatton:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.02

Topic: H.12. Aging and Development

Support: R01 MH109548
R01 MH109548S

Title: Hippocampal contributions to theta in the primary visual cortex

Authors: *J. KENNEDY[1], Y. ZHOU[2], Y. QIN[3], S. D. LOVETT[1], A. SHEREMET[2], S. N. BURKE[1], A. P. MAURER[4];
[1]Neurosci., [2]Engin. Sch. for Sustainable Infrastructure and Envrn. (ESSIE), [3]Civil & Coastal Eng., [4]Evelyn F. McKnight Brain Inst., Univ. of Florida, Gainesville, FL

Abstract: Large amplitude-low frequency oscillations, such as 4-12Hz hippocampal theta, have been consistently shown to entrain faster, lower amplitude oscillations such as gamma. As the local field potentials are primarily shaped by synaptic transmembrane potentials, high-amplitude, low-frequency interactions are thought to serve as a mechanism by which the brain facilitates coordination across regions. As theta has been reported in various cortical and subcortical brain regions, it has been suggested to support a global read-out of synaptic coordination. However, the origin of cortical theta remains the subject of debate. As the hippocampus is one of the largest generators of theta, it has been proposed that cortical theta results from volume conduction from the hippocampus (Winson, 1974; Bland and Whishaw, 1976; Gerbrandt et al., 1978; Sirota et al., 2008; Senzai et al., 2019). Alternatively, others have proposed the hypothesis that cortical theta is locally generated (Zold and Shuler, 2015) or exists as some combination of local generation and volume conduction (Holsheimer et al., 1979; Leung and Borst, 1987). To further explore this hippocampal-cortical interaction, four F344xBN hybrid rats were implanted with a single-shank 64 channel silicon probe in the dorsal hippocampus and primary visual cortex. Visual cortex theta was lower in amplitude than hippocampal theta and exhibited a decrease in coherence further from the hippocampus. Linear cross-region analysis revealed power-power and phase-phase coupling between hippocampal and cortical theta and gamma (50-120 Hz). Bicoherence and cross-region bicoherence were utilized to estimate the magnitude of the nonlinear cross-frequency coupling within the visual cortex and between the visual cortex and hippocampus. Initial results indicate a strong nonlinear phase coupling between theta and its harmonics in the visual cortex and hippocampus. However, no cross-frequency coupling was observed between theta and gamma in the visual cortex. Notably, theta-gamma cross-frequency coupling was observed in mouse visual cortex data generously provided by the Buzsaki laboratory (Senzai et al., 2019). While preliminary results suggest that cortical neurons can

generate theta locally, these data support the hypothesis that cortical theta is, at least in part, the result of volume conduction from the hippocampus.

Disclosures: J. Kennedy: None. **Y. Zhou:** None. **Y. Qin:** None. **S.D. Lovett:** None. **A. Sheremet:** None. **S.N. Burke:** None. **A.P. Maurer:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.03

Topic: H.12. Aging and Development

Support: NIH/NIA 1R01AG049722
NIH/NIA T32-AG061892
Evelyn F. McKnight Brain Research Foundation

Title: Aging and its Effects on Theta Modulation Across Hippocampal Layers

Authors: *N. M. DICOLA[1,2,3], A. L. LACY[4], K. M. KIMSEY[4], J. L. WHITNEY[4], S. D. LOVETT[4], P. TOH[4,5], A. SHEREMET[6], S. N. BURKE[1,2], A. P. MAURER[1,6,2];
[1]Neurosci., [2]Evelyn F. McKnight Brain Inst., [3]Biomed. Sci. Grad. Program, [5]Summer Neurosci. Internship Program, [6]Engin. Sch. of Sustainable Infrastructure and Envrn., [4]Univ. of Florida, Gainesville, FL

Abstract: The theta oscillation (6-12 Hz) is a ubiquitous rhythm found throughout the brain and is related to coordinated synaptic activity across large numbers of neurons. These oscillations are prominent during REM sleep (Graystan and Karmos, 1961) and intentional activity (Vanderwolf, 1969), where they are highly modulated by animal movement speed (Sheremet et al., 2016). Theta in the hippocampus is prominent in the CA1 lacunosum moleculare (LM) and outer molecular layer of the dentate gyrus (OM). Both of these regions have axonal termination sites of the perforant pathway originating from lateral entorhinal cortical layers 3 and 2, respectively. This pathway is degraded in aged rats (Barnes, 1979), non-human primates (Tigges et al., 1995), and humans (Yassa et al., 2010), but the effects of this degradation on animal speed modulation of theta have been under studied. While prior investigations into aging and theta have reported little to no age-related differences (Barnes 1979; Shen et al. 1996; Shen et al. 1997; Abe and Toyosawa, 1999; Orr et al. 2001), these studies were performed using either single wires or tetrodes which do not allow for analyses such as Current Source Density which can be used to localize recording sites based on electrophysiology markers. We therefore implanted male young (4-6 month old) and aged (23-26 month old) Fisher Brown-Norway hybrid rats with linear, 64 channel, 50 µm spaced, silicon probes that spanned 3.15 mm from the pyramidal oriens to the bottom blade of the dentate gyrus. These rats then ran a battery of tasks relating to spatial and

object memory. The aging LM and OM showed significantly increased theta power, even though CA1 pyramidal layer showed no difference. Additionally, theta power in the LM was more heavily modulated by slower velocities in aged rats compared to young. These findings suggest that perforant path degradation has likely caused a compensatory, but more limited, synaptic response in both the LM and OM implying that smaller synaptic drive is required to initiate hippocampal theta in aged rats. This observation may be consistent with the finding that the evoked excitatory post-synaptic population response in the dentate to a given pre-synaptic fiber potential is greater in aged compared to young rats (Foster et al., 1991).

Disclosures: N.M. DiCola: None. **A.L. Lacy:** None. **K.M. Kimsey:** None. **J.L. Whitney:** None. **S.D. Lovett:** None. **P. Toh:** None. **A. Sheremet:** None. **S.N. Burke:** None. **A.P. Maurer:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.04

Topic: H.12. Aging and Development

Support: NIH Grant DA026947
NIMH Grant 1R01MH109548-01A1

Title: Methamphetamine increases cross-frequency interactions in rodent hippocampal LFP and cortical EEG

Authors: *S. D. LOVETT[1], J. P. KENNEDY[1], N. M. DICOLA[1], K. L. ROBERTSON[1], H. KHOSHBOUEI[1], S. N. BURKE[1,2], A. P. MAURER[1,3,4];
[1]McKnight Brain Inst., [2]Inst. on Aging, [3]Biomed. Engin., [4]Engin. Sch. of Sustainable Infrastructure and Envrn., Univ. of Florida, Gainesville, FL

Abstract: A common practice in neurophysiology is to measure the local field potential (LFP) using invasive intercortical electrodes in animal models. The information gained from these methods is often used to advance our understanding of humans, where conclusions are drawn from non-invasive electroencephalogram (EEG) measures. While EEG recordings can also be obtained from rodents, there is a dearth of data relating scalp recordings to the LFP. To imbricate these approaches, we demonstrated how surface EEG can be recorded through skull screws and related to the hippocampal LFP via intracranial silicon probes. Rats were trained to run on a circular track for a food reward. Following baseline recordings under saline administration, rats received I.P. administration of 2.0 mg/kg methamphetamine. Methamphetamine administration resulted in perseverative behavior in which rats traversed the circular track while bypassing the food reward. Furthermore, both the hippocampal and cortical oscillations exhibited a dramatic

increase in power relative to saline conditions. The power of hippocampal theta increased following methamphetamine administration, suggesting that this is a consequence of direct dopamine influence on the hippocampus. However, a more pragmatic explanation is that the effects of methamphetamine are global to the point of increasing total synaptic activity and increasing the mass of synaptic volleys across the brain.

Disclosures: S.D. Lovett: None. **J.P. Kennedy:** None. **N.M. Dicola:** None. **K.L. Robertson:** None. **H. Khoshbouei:** None. **S.N. Burke:** None. **A.P. Maurer:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.05

Topic: H.12. Aging and Development

Support: The McKnight Brain Research Foundation
NIH/NIA 1R01AG049722

Title: Expression of the Immediate-early Genes Arc and Narp during Cognitive Multitasking is Attenuated in Aged Rats

Authors: *C. N. LOGAN, K. N. LUBKE, *S. N. BURKE;
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Abstract: Aged humans (Clapp et al., 2011), monkeys (Gray et al., 2017), and rats are impaired at cognitive multitasking (Hernandez et al., 2015; Hernandez et al., 2019). The working memory/biconditional association task (WM/BAT), which requires the perirhinal cortex (Hernandez et al., 2017), has been used in rats to examine the neurobiological basis of these deficits. For this task, an animal must simultaneously perform a spatial alternation task simultaneously with an object discrimination task in which the correct choice updates based on the animal's position in the maze. Poor performance on this task is related to altered expression of the immediate-early gene Arc in the perirhinal cortex (PER). The expression patterns of other immediate-early genes (IEGs) that may be critical for updating PER cortical circuits in response to behavior, however, have not been examined. The IEG Narp/Nptx2 is a candidate gene that could be disrupted in old age, as this gene is upregulated in response to behavior and its protein is secreted from axon terminals to cluster AMPA receptors on parvalbumin-positive interneurons (Chang et al., 2010; O'Brien et al., 1999). Interestingly, aged rats have reduced monosynaptic coupling between excitatory principal cells and interneurons in the PER (Maurer, Burke, et al., 2017), which could potentially manifest from altered Narp. The current work quantified the co-expression of Arc and Narp in young and aged rats that performed the WM/BAT and a control alternation task. The proportion of cells that were positive for Narp mRNA and co-expression of

Arc and Narp were decreased in aged compared to young rats. These data provide additional evidence that a dysregulation of the balance between inhibition and excitation within the PER contributes to the interruption in feedforward inhibition necessary for cognitive multitasking. Future studies will continue to examine the relationship between the hyperactivation and hypoactivation in cognitive deficits in aging.

Disclosures: C.N. Logan: None. **K.N. Lubke:** None. **S.N. Burke:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.06

Topic: H.12. Aging and Development

Support: NIH/NIA RO1 AG049722
Department of Defense GRANT11811993 for the impact of PERK on post-traumatic tauopathy in Alzheimer's disease
McKnight Brain Research Foundation Institute
UF Summer Neuroscience Internship Program
University Scholars Program

Title: Paired Associates Learning Task as an Indicator of Cognitive Deficit on a Rodent Model of Traumatic Brain Injury

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Abstract: In the United States alone, it is estimated that 1.1 million individuals are treated for Traumatic Brain Injury (TBI) annually. Additionally, 52% of TBI survivors report moderate to severe disabilities 1-year post injury. Animal models of TBI continue to be critical for assessing both the neuropsychological and molecular features of TBI and advancing therapeutic interventions following injury. TBI is characterized by two injury phases. The initial, or acute phase of the injury, leads to tissue loss. The secondary phase of injury is associated with inflammation and molecular cascades that impact neuronal function and can lead to long-term cognitive changes. Currently, preclinical TBI studies rarely characterize injury severity with

clinically relevant cognitive tasks. This study aimed to bridge this gap by using a clinically relevant measure of visuospatial learning and memory on a rodent model of TBI. The task is adapted from the Cambridge Neuropsychological Test Automated Battery (CANTAB), which is used in human clinical settings to assess working memory, learning, and executive function in TBI and dementia. Within CANTAB, we used the paired associates learning (PAL) task, where subjects must associate images with their matched locations on a screen. The development of a touchscreen version of the PAL task for rodents has allowed for increased efficiency and standardization with less input from the researcher. We used a rodent model of controlled cortical impact (CCI) to assess cognitive dysfunction following unilateral parietal (males=4, females= 3) cortical injury or parietal (males=3, females=2) sham craniotomies. Unilateral TBI in the parietal cortex impaired performance on the PAL task relative to sham control animals. Specifically, rats with parietal injury show significantly decreased accuracy on the PAL task and required an increased number of days to reach criterion compared to sham rats. After cognitive testing was completed over several months, quantitative immunohistochemistry was used to evaluate the extent of neuroinflammation as characterized by Iba1 and GFAP immunostaining in affected brain regions. These data indicate that the PAL task could be used to assess cognitive deficits following brain injury. By incorporating the PAL task in models of TBI, we can gain a more complete picture of cognitive outcomes for developing and testing future therapeutic targets.

Disclosures: E.L. Garcia: None. **S.M. Smith:** None. **C.G. Davidson:** None. **N.C. Ferekides:** None. **A. Hernandez:** None. **S.D. Lovett:** None. **J.J. Thompson:** None. **S.R. Malik:** None. **A.V. Bumanglag:** None. **J.F. Abisambra:** None. **S.N. Burke:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.07

Topic: H.12. Aging and Development

Support: The McKnight Brain Research Foundation
NIH/NIA R01 AG049722

Title: The Role of the Dorsal Striatum in Paired-Associates Learning

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Abstract: Despite memory being one of the most frequently studied and well-characterized cognitive domains to date, how different memory systems interact, cooperate, and compete with one another across the lifespan is incompletely understood. This poses a distinct challenge in the

field of cognitive aging, where some systems might be more “vulnerable” or “resistant” than others. It has been hypothesized that there are multiple dissociable systems working in conjunction to support the multifaceted domain of memory. Two of these systems support distinct types of spatial memory. One is supported by the hippocampus and mediates allocentric spatial behaviors. The other is linked to the dorsal striatum and related to response-driven spatial behavior. While both the hippocampus and striatum can support memory-guided behaviors, they are hypothesized to interact antagonistically. Furthermore, aged rats are more likely than young to use striatal-based strategies to solve spatial tasks, and this is related to elevated activity in the dorsal striatum of old animals. Recently is has also been shown that while young rodents perform better on a hippocampal-dependent task than their aged counterparts, aged rodents perform better on a dorsal striatal-dependent task. Interestingly, aged rats that perform comparable to young on a spatial memory task are better able to flexibly switch between tasks that depend on hippocampus or striatum compared to both young rats and aged rats with spatial memory impairment. Together these data suggest that dorsal striatal-dependent memory may either be resilient or compensatory in comparison to hippocampus-dependent memory in advanced age. The role of striatal versus hippocampus-dependent memory systems has not been examined outside of spatial navigation behaviors, however, and it is unclear if the dissociation of age-related vulnerability of different memory systems applies to associative learning. To further investigate the role of dorsal striatum in the context of different memory systems, the current study used an automated, touchscreen-based platform to assess hippocampus-dependent paired associative learning (PAL) after bilateral cannulation and inactivation of the dorsal striatum in rats. Our lab has recently shown that this task is sensitive to age-related cognitive decline. A total of 14 rats were trained to criterion on the PAL task before undergoing cannulation surgery. After surgery, rodents were tested on both PAL and a control T-maze task with infusions of the GABA agonist muscimol. Preliminary data suggests a minimal role for dorsal striatal activity in the retention of acquired visuospatial associations in young rodents.

Disclosures: S.M. Smith: None. **E. Garcia:** None. **S. Lovett:** None. **S.N. Burke:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.08

Topic: H.12. Aging and Development

Support: NIH/NIA RF1AG060977

Title: Effects of long-term ketosis and time restricted feeding on cognitive performance in aged rats

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Abstract: Declining cognitive function is a hallmark of advanced age, reducing quality of life. While caloric restriction can increase lifespan in several species, the data have been equivocal as to whether or not this intervention modifies the cognitive health span. Other interventions such as a ketogenic diet or intermittent fasting have also been shown to increase the lifespan and may be more feasible interventions for enhancing cognitive functioning in old age. We have previously demonstrated that 3+ months of nutritional ketosis improves cognitive function. Furthermore, aged rats fed a ketogenic diet had significantly peripheral metabolic health. Little is known, however, regarding the long-term effects of ketosis during adulthood through advanced age. To investigate this, 8-month old male Fischer 344 x Brown Norway rats were placed on a time-restricted feeding schedule of either a high-fat, low-carbohydrate ketogenic diet (76% fat, 4% carbohydrates, 20% protein) or a calorie equivalent control diet (16% fat, 65% carbohydrates, 19% protein), for 12 months. An additional group was allowed to eat standard rat chow *ad libitum* during this same time frame. After 1 year on the diets, rats were trained on an object-place paired association (OPPA) task and a simple object recognition control task until they reached a criterion performance of 80% correct on two consecutive days. Rats fed ab libitum required significantly more incorrect trials before meeting criterion relative to rats fed via time restricted feeding, regardless of macronutrient composition. Statistically equivalent performance on the control object discrimination task indicated that enhanced cognition on the OPPA task could not be accounted for by differences in motivation, procedural or sensorimotor impairments. Interestingly, there was no significant difference in performance between the ketogenic or control diets, indicating that 12 months of intermittent fasting, with or without carbohydrate restriction, preserves cognitive function later in life. Together these data suggest that mid-life dietary interventions capable of preventing metabolic impairments may also serve to prevent age-related cognitive decline.

Disclosures: C. Watson: None. **A.R. Hernandez:** None. **R. Fletcher:** None. **A. Brotgandel:** None. **A. Ross:** None. **T. Cooper:** None. **J. Thompson:** None. **Q. Federico:** None. **S.N. Burke:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.09

Topic: H.12. Aging and Development

Support: NIH Grant GM120782
the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences grant 0259-2021-0016

Title: Cell type specific differences in prefrontal cortex of young and aging tame and aggressive foxes

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Abstract: Late onset Alzheimer's disease (LOAD) is the leading cause of dementia with over 40 loci having been identified as risk factors. A LOAD-associated gene, *SorCS1*, regulates trafficking of AMPA glutamate receptors and neurorexins and may play a role in APP trafficking and beta-amyloid production. Genetic analysis of tame and aggressive fox populations (*Vulpes vulpes*) developed by 60 years of selection for behavior identified *SorCS1* as one of the main loci implicated in behavioral differences between tame and aggressive foxes. To identify brain transcriptomic differences associated with aging in these populations we performed single nuclei RNA-seq analysis of the prefrontal cortex of six young (7 months of age) and six old (7-9 years of age) female foxes with an equal number of tame and aggressive individuals per group. Sequencing of single nuclei RNA-seq libraries prepared with the Chromium Next GEM Single Cell 3' Library kit (10x Genomics) generated ~498 million reads per sample. The filtered reads were mapped against the dog genome using Cell Ranger 3.0.2 and processed using Seurat. After filtering we identified on average 7,137 cells per sample, 70,396 reads per cell, and 3,878 genes per cell. The clustering analysis identified clusters of excitatory neurons, interneurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells, microglia, and endothelial cells, annotated using known cell type markers. Further clustering revealed multiple subtypes, with the largest subclusters of Layer 2/3 neurons being overrepresented in young foxes and another subcluster of Layer 2/3 neurons and a subcluster of PV interneurons being overrepresented in old foxes. The analysis of differentially expressed genes and Gene Ontology terms in each subcluster revealed larger differences between age groups than between populations. The population specific age-related differences were also identified but the small number of animals in the specific subgroups limited the power to discover and verify these differences rigorously. The analysis of LOAD genes in neuronal subclusters identified 25 genes, including *SorCS1*, significantly upregulated in tame foxes and 35 genes significantly upregulated in aggressive foxes. Two genes (*SorCS3*, *CNTNAP2*) were found to be upregulated in sublusters of Layers 2/3/4 of tame foxes and in a subcluster of Layers 5/6 of aggressive foxes. The well-established fox populations with genetically determined differences in aggressive, affiliative, and anxiety-related behaviors and the life span exciding ten years can provide a new animal model for studying age associated changes in brain functioning and behavior.

Disclosures: A. Kukekova: None. **J.L. Johnson:** None. **E.E. Hecht:** None. **D. Shepeleva:** None. **A.V. Vladimirova:** None. **S.G. Shishkevich:** None. **R.G. Gulevich:** None. **A.V. Kharlamova:** None. **Y.E. Herbeck:** None. **L.N. Trut:** None.

Poster

P895. Learning and Memory in Aging: Animal Models III

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P895.10

Topic: H.12. Aging and Development

Support: AG028271

Title: Post-operative morphine decreases hippocampal synaptic proteins of aged animals during memory consolidation.

Authors: *N. P. DEEMS[1], M. J. BUTLER[1], E. A. SCARIA[1], S. M. MUSCAT[1], K. C. RICE[2], S. F. MAIER[3], G. P. CORTESE[4], R. M. BARRIENTOS[1];
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Abstract: Older adults are more susceptible to cognitive impairments following surgery, and postoperative opioids, such as morphine, can significantly prolong these impairments. Importantly, persistent postoperative cognitive deficits (POCD) are associated with higher risk for dementia, but the mechanisms driving this association are not well understood. In our recently developed rodent model of persistent POCD, we have shown that aged rats that undergo an exploratory abdominal surgery (i.e., laparotomy) followed by one-week of morphine injections (2mg/kg/i.p.) exhibit robust hippocampal-dependent memory impairments lasting at least eight-weeks after surgery. This impairment is associated with many changes in gene expression in whole hippocampus, including increased mRNA expression of synaptophysin and PSD95—two genes which code for proteins highly localized to the pre-and-post synaptic compartments, respectively. To further investigate how synaptic proteins might be impacted in our model, we generated hippocampal synaptoneurosomes from aged, vehicle or morphine-treated animals two-weeks after surgery and performed Western blots to assess relative protein abundance in the synaptic fraction. At baseline there were no significant differences in protein expression of PSD95, synaptophysin, and subunits of the AMPA and NMDA receptors. However, 2 hours post-learning (context preexposure), marked differences between groups were observed. Vehicle-treated animals demonstrated robust increases in levels of synaptic proteins while morphine-treated animals exhibited profound decreases compared to those at baseline. These data suggest that the combination of aging, surgery, and morphine disrupts the proper

abundance and coordination of synaptic proteins necessary for the formation of memory in the hippocampus.

Disclosures: N.P. Deems: None. **M.J. Butler:** None. **E.A. Scaria:** None. **S.M. Muscat:** None. **K.C. Rice:** None. **S.F. Maier:** None. **G.P. Cortese:** None. **R.M. Barrientos:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.01

Topic: H.12. Aging and Development

Title: Anti-diabetic effects of chaya Cnidoscolus chayamanasa in type 2 diabetes mouse model TallyHO

Authors: *B. RAMASUBRAMANIAN[1], M. HANSON[3], C. GRIFFITH[3], P. H. REDDY[2]; [1]Intrnl. Med., [2]Dept. of Intrnl. Med., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [3]Texas Tech. Univ., Lubbock, TX

Abstract: The purpose of our study is to understand the protective effects of Chaya (*Cnidoscolus chayamansa*) leaf extract against type 2 diabetes (T2D). Type 2 diabetes accounts for 90 to 95% of all diabetic patients. Diabetes is highly prevalent in elderly populations and is a major health concern. Increasing evidence suggests that T2D is a major factor for developing late-onset Alzheimer's disease (AD), although a direct link between T2D and AD has not been understood well. Chaya is an edible, spinach-like medicinal plant native to Central America that has been believed to have anti-diabetic properties. Chaya is believed to cure diabetes and other cardiovascular diseases. To date, very few laboratory experiments on Chaya have been conducted, yet all of them have shown Chaya to have some degree of antihyperglycemic effects. **Methods:** In the current study, we have used a polygenic mouse model for T2D, the TallyHo (TH) mice to elucidate the anti-diabetic effects of Chaya. Only male TH mice develop hyperglycemia, hyperinsulinemia, hyperlipidemia, moderate obesity, and enlargement of the islets of Langerhans at the age of 16 weeks of age. Female mice display moderate hyperinsulinemia, hyperlipidemia, and obesity but do not manifest overt diabetes. We used Chaya leaf extract, mixed with regular chow as a diet supplement. Our preliminary study indicates that glucose levels were maintained in Chaya-fed fully diabetic 12 month-old TH mice compared to control TH mice fed with regular chow. In addition, Chaya Chow fed TH male mice showed significantly reduced body weight compared to regular Chow Fed TH male mice. **Results:** Our results also indicate that the expression for mitochondrial dynamics proteins, Drp1 and Fis1 show a significant increase in the diabetic mice (TH) compared to the non-diabetic control mice. However, the protein expression is comparable to the non-diabetic control mice in the Chaya treated TH mice. Our results also show a significant decrease in the fission proteins,

autophagy, mitophagy proteins, biogenesis and synaptic proteins in the TH mice compared to the non-diabetic control mice. The expression of proteins in the Chaya treated TH mice are equivalent to the non-diabetic control mice. **Conclusions:** Findings from our study suggests that *Chaya* protects against hyperglycemic effects and improves mitochondrial biogenesis, autophagy and mitophagy in TH mice.

Disclosures: B. Ramasubramanian: None. **M. Hanson:** None. **C. Griffith:** None. **P.H. Reddy:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.02

Topic: H.12. Aging and Development

Title: Chronic high sucrose diet in early stages induced impairment cognition in spatial memory in young Wistar rats

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Abstract: The consumption of sugar-sweetened beverages (SSBs) has increased over the past 30 years; in adolescent and young adult this SSBs apport 15% of the calories of their diet and are strongly associated not only with the development of obesity and metabolic diseases but also with cognitive impairment. In particular negative effects have been reported in hippocampal dependent task such as spatial memory when chronic but restricted SSBs are given in adult animal models. The effects of acute stimuli in critical stages as puberty in addition to chronical and continous SSBs should be discussed.The aim of study is to analyse if spatial memory impairment is observed in the BM after the continuous consumption of SSBs from postnatal day 21 (p21) to young adulthood, and if this impairment persists until 3 months of age with long-lasting SSBs consumption; furthermore we observe if an acute LPS administration during prepuberal phase (p37) could enhance the effects of the SSBs consumption in the acquisition and retention of the task. We used 18 male Wistar rats postweaded (p21) fed with standard chow, SSBs (sucrose 35%) ad libitum for the High Sucrose Diet group (HSD). For the High Sucrose Diet with LPS group (HSD-LPS) standard chow and SSBs ad libitum and a unique dose of Lipopolisacharid (LPS) 1.5mg/kg intraperitoneal (i.p) at p37 was given, and finally, the control group (CTRL) took standard chow and water ad libitum. Each group was assessed at p54, p81, p101 with a BM and latency escape was measured. At the acquisition training (At) 7 trails were

taken and 24hrs after the adqusition probe (Ap) and recovery probe (Rp) phase was taken. During the experiment both diets showed significant lower weights compared to the CTRL group, glucose levels increased significantly at the second week of the DHS group, but DHS-LPS increased their glucose levels until the sixth week. In BM task. DHS-LPS group took more time in the latency escape at the first sessions when the At was evaluated. For the Ap the latency seems significantly higher for both groups during the first 2 sessions and finally the latency escape during the Rp was higher during the first session for both groups. We conclude for instance that both treatments affect the spatial memory since they took more time on trails for the acquisition of the task comparing to the CTRL group. Also, the maintenance of the task in the memory (24h) was affected in both groups, eventhough it seems more severe for the DHS-LPS group than for the DHS group. This could be related with the anxiolytic effect that beverages rich in sugar could cause when acute stressors are presented, but no when the diet is the stressor itself.

Disclosures: A. Llanes-duran: None. **G.E. Aranda-Abreu:** None. **M.E. Hernández-Aguilar:** None. **J. Manzo-Denes:** None. **D. Herrera-Covarrubias:** None. **C.A. Pérez-Estudillo:** None. **F. Rojas-Duran:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.03

Topic: H.12. Aging and Development

Support: National Institute of General Medical Sciences / National Institutes of Health, grant R01GM128183
College of Veterinary Medicine.

Title: Genetic ablation of dentate hilar somatostatin-positive GABAergic interneurons is sufficient to induce cognitive impairment

Authors: *R. NAGARAJAN[1], J. LYU[1,2], M. KAMBALI[1], M. WANG[1,2], C. D. COURTNEY[3,2], C. A. CHRISTIAN-HINMAN[3], U. RUDOLPH[1];
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Abstract: Aging is often associated with a decline in cognitive function. The molecular and cellular mechanisms underlying these cognitive impairments remain poorly defined, but mounting evidence points to changes in GABAergic function as a major causal factor. Interestingly, a reduction in the number of somatostatin-positive (Sst+) interneurons in the DG has been described in aged cognitively impaired but not in unimpaired rodents. However, it

remains unclear whether the reduction in Sst+ interneurons in the DG hilus is directly related to or even causal for age-related cognitive dysfunction. We hypothesized that hilar Sst+ interneurons play an essential role in maintaining cognitive function and that a reduction in the number of hilar Sst+ interneurons might be sufficient to induce cognitive dysfunction. Hilar Sst+ interneurons were ablated by expressing a diptheria toxin transgene specifically in these interneurons. An AAV-mCherry-flex-dtA construct with the EF1α promoter was stereotaxically injected (bilaterally) into the DG hilus of young adult Sst-Cre mice (2-3 months). The control group was injected with an AAV-mCherry virus. After 21 days, the mice were employed for the novel object recognition test (CA1-dependent), the Morris water maze with reversal learning (learning: CA1- and CA3-dependent; reversal learning: DG-dependent), and a Y-maze test (HP, PFC). Brain tissue was collected for immunohistochemistry (c-Fos, GAD-67, Sst). We found that partial genetic ablation of Sst+ hilar interneurons impaired learning and memory functions as determined in the behavioral test battery. It also significantly increased the number of c-Fos-positive neurons in the DG and CA3 regions. Moreover, the number of Sst+ interneurons in the DG was decreased by 49 %, and the number of GAD-67+ neurons 33%. Our results show that the partial ablation of hilar Sst+ interneurons is sufficient to induce changes similar to those observed in aged rodents, such as reduction of Sst in the DG hilus, hyperactivity in DG and CA3 of the hippocampus, and cognitive impairments. This suggests that the changes elicited by partial ablation of hilar Sst+ interneurons may contribute to the development of cognitive dysfunction in aging animals. Furthermore, mice with a partial ablation of hilar Sst+ interneurons may represent a model for studying mechanisms underlying cognitive decline in aging.

Disclosures: R. Nagarajan: None. **J. Lyu:** None. **M. Kambali:** None. **M. Wang:** None. **C.D. Courtney:** None. **C.A. Christian-Hinman:** None. **U. Rudolph:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.04

Topic: H.12. Aging and Development

Support: NIH KO8NS096115
NIH 3K08NS096115-03S1
JHU-SOM Clinician Scientist Award

Title: Late deficits in PV+ interneurons, E/I balance, and mechanism of synaptic plasticity in dorsal hippocampus linked to neurobehavioral abnormalities after neonatal hypoxic-ischemic brain injury

Authors: P. PARMAR[1], N. RASTOGI[1], S. MILLER[1], C. LECHNER[1], M. ST. PIERRE[1], H. SPAHIC[1], A. KIRKWOOD[2], *R. CHAVEZ-VALDEZ[1];
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Abstract: Infants suffering of hypoxic-ischemic (HI) brain injury have a higher risk for neurobehavioral deficits despite therapeutic hypothermia (TH). Neonatal HI decreases the number of hippocampal parvalbumin $(PV)^+$ interneurons (INs) in the mouse. Whether deficits persist to adulthood leading to excitatory/inhibitory (E/I) and behavioral disturbances is still unknown. We hypothesized that neonatal HI results in deficits in hippocampal PV^+INs, E/I balance, and mechanisms of synaptic plasticity, which correlate with behavioral deficits. We induced brain HI in P10 C57BL6 mice with R carotid ligation + 45m of hypoxia (8%), and then randomized to normothermia (36ºC, NT) or TH (30ºC) for 4h with anesthesia-shams as controls. Number and morphology of PV^+ INs in the dorsal hippocampus were assessed using IHC. E/I disturbance was assessed using puntae quantification for VGlut2 to SYT2. Images were processed using Zenlite, ImageJ/FIJI, and IMARIS software. Extracellular field potentials (EFP) were performed to assess LTD and LTP in Shaffer collateral synapses at P18 and P40. Behavioral testing, which included open field (OF), object location test (OLT), and Y-maze testing was processed using AnyMaze software. At P40, the number of PV^+ interneurons was decreased by 47% and 42% ($p<0.05$) in the dorsal hippocampus of injured mice treated with NT or TH, respectively. These deficits were similar in both sexes. GFAP score correlated well with residual hippocampal volume at P40 ($p<0.001$). Dendritic simplification of PV^+ interneurons 30 days after HI was documented by the decreased number of branches (44%), segments (48%), braching points (45%) , and sholl intersections (30%). Ratios of Vglut2/SYT2 punctae count, volume and intensity, were increased by 4-fold, 5-fold, and 5.5-fold, respectively, at P40. TH did not prevented any of these disturbances. At both P18 and P40, LTD and LTP were impaired using EFP in dorsal Schaffer collateral synapse ($p<0.001$). The decreased time spent in the center of the arena (OF), in novel arm (Y-maze), and exploring the novel object (OLT) suggested impaired memory and increased anxiety after neonatal HI, which correlated with decreased number of PV^+ INs in the dorsal hippocampus. Neonatal HI produces a long-lasting deficit in PV^+ interneurons in the dorsal hippocampus, which is independent to severity of injury, and is not attenuated by TH. This deficit results in disturbed E/I balance towards excitation, impaired mechanisms of synaptic plasticity and correlates with memory deficits and anxiety-like behavior. Persistent GABAergic deficits may explain neurobehavioral anomalies described in patients surviving neonatal HI encephalopathy.

Disclosures: P. Parmar: None. **N. Rastogi:** None. **S. Miller:** None. **C. Lechner:** None. **M. St. Pierre:** None. **H. Spahic:** None. **A. Kirkwood:** None. **R. Chavez-Valdez:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.05

Topic: H.12. Aging and Development

Support: NIH Grant AG062968

Title: Inbred WMI rat strain as a genetic model for mild cognitive impairment

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Abstract: The link between Alzheimer's Disease-related dementia and mild cognitive impairment (MCI) is an area of interest with growing possibilities. MCI can progress to Alzheimer's Disease (AD) and this path is vulnerable to specific risk factors. Stress and stress-related disorders, like major depressive disorder (MDD), have been associated with accelerated cognitive aging. Despite the significance of this issue, naturally occurring MCI models are very scarce. Thus, an animal model that shows premature memory loss, and is genetically characterized, could be a very useful tool for drug discovery. Our genetic model was developed from the Wistar Kyoto (WKY) rat strain, with depression and anxiety-like behaviors. Two fully inbred strains were generated with opposite behavior in the FST: the WKY More Immobile (WMI), and the WKY Less Immobile (WLI) strains. Middle-aged (12 months old) WMI females showed a 75% reduction of fear memory from the young adult (6 months old) WMI females' freeze duration in the contextual fear conditioning (CFC) (age, $F[1,30]=25.17$, $p<0.01$; age x strain, $F[1,30]=16.25$, $p<0.01$); no age effect was seen in the males (age, $F[1,75]=0.53$, NS). However, in the hippocampus-dependent spatial memory task, the Morris Water Maze (MWM), both middle-aged WMI males and females showed significantly lower levels of memory as measured by latency to reach the platform compared to WLIs (females: strain, $F[1,10]=9.07$, $p=0.01$; males: strain, $F[1,10]=30.50$, $p<0.01$; strain x day, $F[3,30]=7.67$, $p<0.01$). Middle-aged WMI females showed consistently lower levels of learning (Day 1) and memory (Day 4) compared to WLIs, while WMI males consistently missed the platform, being unable to remember the orientating signs on Days 3 and 4. The CFC and the MWM assess hippocampus-dependent fear and spatial working/reference memory, respectively, which are highly sensitive to aging. There were no differences in these measures between young WLI and WMI animals. Interestingly, CFC is thought to be not as sensitive to aging as other hippocampus-dependent tasks. Perhaps this explains why spatial memory decline can be detected in both middle-aged WMI males and females in the MWM but only in females in the CFC. Alternatively, strain and sex-specific expression differences found in the hippocampus of middle-aged animals in sex hormone receptors can contribute to the sex-differences in CFC and in the pattern of MWM at middle age.

Disclosures: C. Kim: None. **K.J. Przybyl:** None. **E.E. Redei:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.06

Topic: H.08. Learning and Memory

Support: Jordan's Guardian Angels
NIH Grant MH115673
NIH Grant MH113352
NIH Grant DK116624
Roy J. Carver Charitable Trust
Iowa Neuroscience Institute

Title: Generation and characterization of mice harboring Jordan's Syndrome alleles causing intellectual disability with autism

Authors: *R. MERRILL[1], C. JONG[2], C. CHEN[2], P. SANDAL[2], G. HEALEY[2], Y. KONG[2], S. STRACK[3];
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Abstract: BACKGROUND: Protein phosphatase 2A (PP2A) is an abundant and essential serine/threonine phosphatase in eukaryotic cells. Much of PP2A's specificity is directed through the different regulatory subunits, and many of these regulatory subunits have recently described mutations associated with a wide variety of human diseases ranging from cancer to intellectual disability. To date, there are more than two hundred individuals identified with Jordan's Syndrome, a disease characterized by the presence of *de novo* mutations in the *PPP2R5D* gene, which encodes the PP2A regulatory protein B'delta. The affected individuals' symptoms vary depending on the specific allele but can include intellectual disability, language delay, autism spectrum disorder, and seizures.
METHODS: The frequency and severity of the disease-causing mutations varies in humans but most are negative to positive charge-reversal mutations. To investigate phenotypes and develop animal models of the Jordan's Syndrome, we generated three mouse lines harboring mutations in *Ppp2r5d* using CRISPR-Cas9 gene editing at the University of Iowa Genome Editing Core Facility. Two mutations, E198K and E200K, are in contained in a highly conserved loop but have differing symptoms in humans, with the E198K being more severe. The third mutation, E420K, is further away on the primary protein sequence, but patients with this allele also display severe symptoms.
RESULTS: Mice heterozygous for any of these mutations are viable, but pups with E198K or E420K are born below the expected frequency. Additionally, the E198K and E420K mice often die as juveniles and show craniofacial abnormalities including cranial bossing as determined by CT scans. We have performed a battery of behavioral tests, and the mice show hyperactivity and learning memory deficits that vary with the specific mutation but correlates well the severity observed in humans. Lastly, all three mouse lines demonstrate spontaneous and handling induced seizures.

CONCLUSION: Mice heterozygous for the *Ppp2r5d* mutations (reflecting the condition of the patients) have phenotypes that correspond with the severity observed in Jordan's Syndrome patients. These generated mouse lines represent faithful models for studying the human disease.

Disclosures: R. Merrill: None. **C. Jong:** None. **C. Chen:** None. **P. Sandal:** None. **G. Healey:** None. **Y. Kong:** None. **S. Strack:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.07

Topic: H.08. Learning and Memory

Support: Orphan Disease Center's MDBR Pilot Grant Program

Title: Tcf4 reinstatement in glutamatergic neurons rescues cognitive deficits in a mouse model for Pitt-Hopkins Syndrome

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Abstract: *Tcf4* is a transcription factor that is expressed in high levels in neural tissue and its haploinsufficiency is the cause of an autism-spectrum known as Pitt-Hopkins Syndrome. The deletion of one *Tcf4* gene copy in mice causes severe cognitive deficits. Here we utilize a lox-stop-lox *Tcf4* mouse model that allows for Cre-dependent reinstatement of gene function. After assessing different cell populations, it was found that the reinstatement of *Tcf4* in glutamatergic neurons post-developmentally rescued learning and memory phenotypes measured by object location memory and the Morris water maze. These data suggest that a gene therapy targeting glutamatergic *Tcf4* reinstatement post-developmentally may be efficacious to treat the cognitive deficits found in Pitt-Hopkins Syndrome.

Disclosures: S.M. Strugar: None. **M.M. Bishop:** None. **M.M. Caron:** None. **A. Howard:** None. **X. Zhang:** None. **B. Malachowsky:** None. **A.J. Kennedy:** None.

Poster

P900. Cognition: Animal Models

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P900.08

Topic: H.08. Learning and Memory

Support: NIH/NINDS 3R01NS089578-03S1

Title: Dendritic spine abnormalities and behavioral deficits in forebrain specific Par1c/MARK1 knockout mice

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Abstract: Dendritic spines are dynamic postsynaptic structures that play an important role in cognitive functions, such as learning and memory. The dysregulation of spine size, shape and density may lead to learning and memory deficiencies, as well as neurological disorders such as autism spectrum disorders (ASD). Previous studies from our laboratory have demonstrated that partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), regulates dendritic spine morphogenesis and plasticity in cultured hippocampal neurons. Interestingly, studies have found multiple single nucleotide polymorphisms of MARK1 associated with ASD and bipolar disorder. However, the role of Par1c/MARK1 in synaptic plasticity and cognitive functions *in vivo* is still unknown. Therefore, we developed a conditional knockout (cKO) MARK1 mouse model to examine the effects of MARK1 depletion on spine morphogenesis and cognitive functions such as learning and memory. In this mouse model, MARK1 is depleted postnatally from pyramidal neurons of the forebrain including the hippocampus and the cerebral cortex. The MARK1 conditional KO mice show a significant decrease in dendritic spine density, width, and length in the hippocampal CA1 region compared to wild-type controls. In addition, there is an increase in stubby spines and a reduction in mushroom-shaped spines. We also studied different synaptic proteins levels and found increased synaptic levels of GluR2 in the MARK1 cKO mice. Furthermore, MARK1 cKO mice show a defect in spatial learning in the Morris water maze test and significantly reduced anxiety in the elevated plus-maze. Proteomic analyses show MARK1 targets synaptic proteins as well as proteins regulating cytoskeletal dynamics and vesicle trafficking. Specifically, there is a significant decrease in the phosphorylation at residue S797 of RalGAPα1, which is a GTPase-activating protein (GAP) for Ral GTPases. Since Ral GTPases regulate membrane trafficking, current studies are aimed at examining whether MARK1 regulates receptor trafficking through RalGAPα1. Together, our studies point to an important role for Par1c/MARK1 in regulating dendritic spine morphogenesis, spatial learning and anxiety-related behavior *in vivo*.

Disclosures: E.C. Kelly-Castro: None. **M. Sun:** None. **H. Zhang:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.01

Topic: I.03. Anatomical Methods

Support: NIDA U01DA043098
Office of Naval Research (ONR) 00014-19-1-2149
The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium

Title: Effect of different organic fluorophores on signal quality and multiplexing ability of fluorescence in situ hybridization chain reaction method

Authors: *V. KUMAR, D. M. KROLEWSKI, A. PARSEGIAN, M. FOLTZ, B. MARTIN, H. AKIL, S. J. WATSON, Jr.;
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Abstract: Fluorescence in situ hybridization chain reaction (HCR FISH) offers one of the most efficient and simple methods of signal amplification for transcript probing in varied technological settings including thin section as well as large intact tissues (Choi et. al., 2014, 2018, Sylwestrak et. al., 2016, Kumar et. al., 2021). Applying multiple custom designed split-pair probes, and fluorophore-labeled DNA hairpins to tissue concurrently allows for multiplexing of up to five targets with high specificity. Consistent fluorescence signal with optimum signal to noise (S/N) ratio is a determining factor for both qualitative and quantitative analysis, especially dependent upon the colocalization. Through our repeated studies using thin sections (10-30 µm) as well as iDISCO$^+$ processed large rodent brain tissues (few mm thick slices to intact hemispheres), we discovered that effectiveness of the HCR FISH technique was critically dependent on the choice of organic dyes used as fluorophores. For example, we consistently found that hairpins conjugated with AlexaFluor-647 (AF-647) produced the most reliable fluorescence signal with highest S/N ratio and proved to be the most resilient to buffers and mounting mediums as well as photobleaching under extended imaging sessions using both confocal and light sheet microscopes. In contrast, hairpins tagged with AF-405/-488/-594 often failed to produce as consistent and comparable S/N ratios across the experiments, however it was dependent upon the target gene and tissue processing method. The fluorophore-based differences were most evident when HCR FISH was performed on low copy number genes using perfused and or iDISCO$^+$ cleared brain samples. Next, we tested DNA hairpins conjugated with non-AF fluorophores, FITC, Cy3, JaneliaFluor-525 and BODIPY-590, but these dyes too failed to produce a comparable signal to that of AF-647. Here, we report and discuss above-mentioned findings and their potential mechanisms. Then, we explore the future directions to establish the exact cause of such fluorophore-based differences in signal output and alternate solutions to achieve adequate and consistent fluorescence for multiplexed labeling and benefit both qualitative as well as quantitative studies.

Disclosures: V. Kumar: None. **D.M. Krolewski:** None. **A. Parsegian:** None. **M. Foltz:** None. **B. Martin:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.02

Topic: I.03. Anatomical Methods

Support: NIH Grant U24 NS109113

Title: Generation of recombinant monoclonal antibodies and scFvs against brain targets using sequences derived from high-throughput hybridoma sequencing

Authors: *J. S. TRIMMER, J. BERNAL, H. N. FRIZZO, C. GAVIRA-O'NEILL, B. GONG, K. D. MURRAY, K. M. TEMPELTON, D. VAN DER LIST;
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Abstract: We previously developed a widely-used collection of monoclonal antibodies (mAbs) targeting brain proteins. These mAbs have been extensively validated in essential neuroscience applications including immunoblots and immunohistochemistry on mammalian brain samples (neuromab.ucdavis.edu). We are now converting this hybridoma-based mAb collection into recombinant form (i.e., into recombinant mAbs or R-mAbs) to create molecularly-defined research reagents. Conversion into recombinant form will also allow for enhanced archiving and dissemination as plasmids and/or DNA sequences, more reliable expression, and will allow for diverse forms of antibody engineering. Our initial efforts (Andrews et al., 2019, eLife 8: e43322) employed cloning of RT-PCR amplified heavy and light chain variable (V_H and V_L) domain sequences into a mammalian expression plasmid, followed by sequencing of V_H and V_L domains. We have more recently employed a high-throughput Illumina-based sequencing platform to sequence the V_H and V_L domain sequences directly from hybridomas, and are posting these sequences on the NeuroMabSeq website (neuromabseq.ucdavis.edu). We have now used these V_H and V_L domain sequences to develop R-mAb expression plasmids. We synthesize gene fragments corresponding to V_H and V_L domain sequences, and use Gibson Assembly to insert them into a mammalian expression plasmid containing all other elements needed for expression of R-mAbs, while engineering an IgG subclass switch to facilitate multiplex labeling. We transfect these plasmids into mammalian cells to generate R-mAb samples for validation relative to the hybridoma-derived mAbs. We use an analogous approach to engineer mammalian expression plasmids for miniaturized single chain variable fragments (scFvs). We synthesize V_H -linker- V_L domain gene fragments and use these in Gibson Assembly reactions to generate scFv expression plasmids. Due to their smaller size, these scFvs have enhanced tissue penetration and yield higher resolution imaging than conventional antibodies. We have partnered with Addgene to make these R-mAb and scFv plasmids and sequences publicly available

(https://www.addgene.org/James_Trimmer/). Together, these efforts will provide the community with open source, renewable, recombinant research reagents to support neuroscience research.

Disclosures: J.S. Trimmer: None. **J. Bernal:** None. **H.N. Frizzo:** None. **C. Gavira-O'Neill:** None. **B. Gong:** None. **K.D. Murray:** None. **K.M. Tempelton:** None. **D. Van Der List:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.03

Topic: I.03. Anatomical Methods

Title: Resolving Spatial Gene and Protein Expression in the FFPE Mammalian Central Nervous System

Authors: A. D. HARTNETT, P. MIELINIS, ***C. R. UYTINGCO**, J. CHEW, F. MESCHI, R. CHAN, C. GALONSKA, A. JUREK, L. KATIREAEE, T. CHEN, D. MORALES, M. TURKKUL, T. DRENNON, S. R. WILLIAMS, P. ROELLI, N. WEISENFELD, F. BAUMGARTNER, J. STERN, K. R. MILLER, D. WALKER, Y. YIN, M. STOECKIUS, C. GALLANT, J. CHELL;
10X Genomics, Pleasanton, CA

Abstract: Understanding the detailed cellular composition and gene expression of the mammalian central nervous system (CNS) is critical for gaining insights into normal, developing, and diseased states. In addition, correlating transcriptional changes with translational profiles provides a more holistic view of the underlying CNS biology. Despite the sensitivity of gene expression measurements from RNA-seq, the loss of tissue structure with the inability to examine protein expression limit the technology. The recently introduced 10x Genomics Visium Spatial Gene Expression Solution enables the use of histological techniques together with the highly multiplexed identification of RNA molecules to spatially map the gene expression patterns in FFPE samples. Visium for FFPE incorporates ~5,000 gene expression spots per sample area on a slide, wherein each spot has spatially barcoded primers over which tissue is placed. The tissue sections are subsequently H&E stained or immunostained, imaged, and then permeabilized, allowing for the detection of native RNA. In the present experiment, whole mouse and human transcriptome probe panels were used to capture the transcriptional profiles of the respective CNS tissues. Libraries were generated and sequenced and the resulting RNA-seq data were mapped back to image coordinates, placing gene expression into context within the H&E or immunostained tissue image. The data were integrated into the 10x Genomics Space Ranger analysis pipeline in which a single section whole transcriptome readout and the reference image were correlated and subsequently visualized using the 10x Genomics Loupe Browser desktop software. By aggregating proteomic and transcriptomic data from immunostained

sections, the resolution of cell-type identification was improved. Results from these efforts suggest that this multiomics approach can provide a powerful complement to traditional histopathology, enabling a greater understanding of cellular heterogeneity and organization within the mammalian CNS. Furthermore, this more detailed view of the mammalian CNS anatomy can provide essential insight into the cell type-specific nature of neurobiology and neurodegenerative diseases.

Disclosures: A.D. Hartnett: A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **P. Mielinis:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **C.R. Uytingco:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **J. Chew:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **F. Meschi:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **R. Chan:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **C. Galonska:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **A. Jurek:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **L. Katireaee:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **T. Chen:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **D. Morales:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **M. Turkkul:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **T. Drennon:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **S.R. Williams:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **P. Roelli:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **N.**

Weisenfeld: A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **F. Baumgartner:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **J. Stern:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **K.R. Miller:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **D. Walker:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **Y. Yin:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **M. Stoeckius:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **C. Gallant:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics. **J. Chell:** A. Employment/Salary (full or part-time):; 10X Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10X Genomics.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.04

Topic: I.03. Anatomical Methods

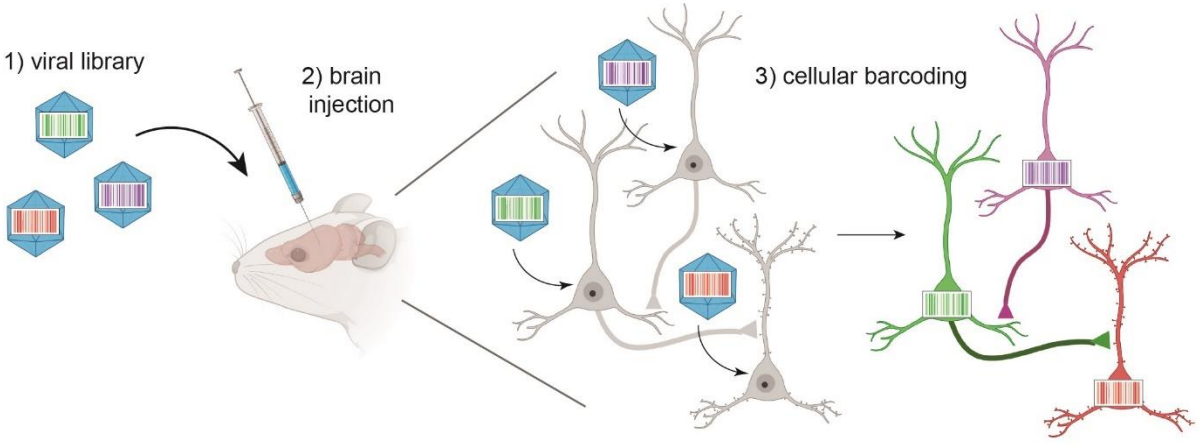
Support: NIH/NINDS grant 1R01NS112716-01A1
IARPA contract D16PC0008

Title: Progress toward megaplexed cellular barcoding of the mouse brain

Authors: T. LEBAR[1,2], I. HAN[1], N. ARKAN[1], G. M. CHURCH[1,2], ***R. E. KOHMAN**[1,2,3]; [1]The Wyss Inst. at Harvard, Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]The Wyss Ctr. for Bio and Neuroengineering, Geneva, Switzerland

Abstract: A basic understanding of how individual cells within the brain operate and organize to give rise to sensations, behavior, and thoughts is lacking. This knowledge gap is the result of the staggering complexity of the central nervous system. Each neuron sends and receives local and

long range connections through cellular projections which can often travel along complex paths. Mapping these connections with single-cell resolution would be an immense step forward for neuroscience, however no technology exists that can achieve both the necessary scale and resolution, as a tradeoff generally exists between these two. Fluorescence microscopy is potentially best suited to map neural circuitry in mammalian brains because it can image thick tissues at resolutions necessary to distinguish fine cellular processes such as dendritic spines. Its resolution limit becomes problematic however when imaging densely labelled tissue because overlapping features of cells cannot be distinguished. The use of multiple fluorescent colors or color combinations improves this situation, but not enough to map dense circuits over long ranges. Here we will discuss advances in the writing and reading of cellular barcodes in mammalian brain tissue. This approach involves virally labelling cells with nucleic acids or peptide identifiers in the mouse brain. Because of the high sequence diversity of these molecules, millions of "virtual colors" are useable for labelling cells rather than a small color palette of fluorophores. This megaplexed labelling strategy can potentially scale to label all cells within mammalian brains and lead to high throughput circuit mapping over large distances.



Disclosures: T. Lebar: None. **I. Han:** None. **N. Arkan:** None. **G.M. Church:** None. **R.E. Kohman:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.05

Topic: I.03. Anatomical Methods

Support: NINDS Grant R01NS111028
NIH Brain Initiative Grant 1RF1NS113287

Title: Microcomputed tomography and computer vision based morphometric analysis pipeline for characterizing anatomical variations in skull morphologies in wildtype and transgenic mice strains

Authors: *B. R. GULNER, Z. S. NAVABI, B. VANHEEL, S. B. KODANDARAMAIAH; Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: The anatomy of the skull and the brain of commonly used laboratory mice strains, such as the C57/BL6 strain have been exhaustively characterized, and precise anatomical atlases of brain have been constructed. While these results indicate a well conserved brain anatomy within standard strains, much less is known of any variations in size and shape of brain and skull of genetically engineered transgenic mice derived from the C57/BL6. A precise characterization of variations in skull morphologies, and in turn brain morphology, may enable more accurate targeting of deep brain targets during stereotactic surgery, and a comprehensive mapping of skull thickness variations may improve guiding of automated cranial microsurgeries. We have developed a high throughput computer-vision based analysis pipeline for analyzing microcomputed tomography (µCT) scans of skulls of mice to evaluate geometric morphometrics and skull thickness. We performed µCT scans on 5 male and 5 female C57/BL6 mice at 15 weeks age procured from a commercial vendor (Jackson Laboratories) and on 4 female in-house bred Thy1-GCaMP6f mice at 16 weeks age. A custom MATLAB program was used to measure the thickness of the dorsal skull in the resulting µCT image stack. Eleven landmarks were used to automatically register each skull to an average reference skull using piecewise linear transformations. The landmark locations were similar across sex and strain, indicating that the gross morphology of the skull was conserved. Thickness of the dorsal skull in three regions of interest (ROIs) comprising the parietal, frontal, and interparietal bones lateral to the midline were measured. We found that the male C57BL/6J mice had significantly thicker skulls than the female C57BL/6J mice in the parietal and frontal ROIs ($p=0.002$ for both regions, t-test). The mean thickness of the interparietal ROI did not vary significantly between the male and female C57BL/6J mice ($p=0.6$). The female C57BL/6J mice had thicker skulls than the 16-week female Thy1-GCaMP6f mice in all three ROIs ($p=0.01$ parietal; $p=0.03$, frontal; and $p=1e-4$, interparietal ROI). We are currently performing similar analysis on other in-house strains and sexes of mice using this pipeline and extending our analysis to geometric morphometrics. Our results indicate strain- and sex-specific differences which need to be considered when planning stereotactic cranial microsurgery. In the future, detailed maps of skull anatomy and thickness may assist automated cranial microsurgeries. The method used for automatically extracting bone thickness from µCT scans also may be widely useful for efficiently measuring dimensions of radiopaque features in µCT scans.

Disclosures: B.R. Gulner: None. **Z.S. Navabi:** None. **B. VanHeel:** None. **S.B. Kodandaramaiah:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.06

Topic: I.03. Anatomical Methods

Support: NIH Grant MH117820

Title: A pipeline for high-resolution cellular and pathologic atlases in human brains

Authors: *E. TURSCHAK[1], P. BALARAM[1], K. TAKASAKI[1], N. POSTUPNA[2], E. PETERSON[1], A. EDGREN[1], N. OUELLETTE[1], B. MACLENNAN[1], M. TAORMINA[1], A. GLASER[3], J. WATERS[1], C. D. KEENE[2], R. C. REID[1];
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Abstract: Comprehensive cytoarchitectonic atlases that visualize areal, laminar and cellular diversity in human brains are invaluable to the study of human brain disease, since they provide anatomical descriptions of brain areas under normal conditions that serve as a reference for pathological changes in disease states. However, most current atlases are limited in scale and resolution; volumetric atlases from resonance imaging data span whole brains but cannot visualize laminar or cellular diversity, while histological atlases from light microscopy data reveal fine structure but are typically limited to serial sections <100um. Recent advances in hydrogel-based tissue clearing methods and light sheet microscopy enable the generation of high resolution cytoarchitectonic data in large volumes of cleared tissue.
Human brain tissue is difficult to clear, fluorescently label, and image in large volumes (>300 um) due to the density of the white matter and the typically high levels of fixation for archived human specimens. These issues have posed barriers for the development of high-resolution cellular and pathological maps of human brains. Advances in tissue clearing methods have helped mitigate these problems, but to our knowledge have not been used so far to create large-scale volumetric atlases, which would exceed 1 petavoxel for a human brain imaged at 1 micrometer resolution.
We have employed the ELAST protocol (Ku et al, Chung 2020 Nature Methods) because it adds structural stability to brain tissue while enabling faster clearing and increased label penetration throughout thick volumes. We subjected cleared tissue-hydrogel matrices to cytoarchitecture-specific stains to fluorescently label nuclei, cell bodies, and vasculature. We then used light-sheet fluorescence microscopy to image these tissue volumes at high speed but modest (~1 micrometer) resolution. We have thus built a pipeline for efficiently creating high-resolution cytoarchitecture maps. By applying specific pathological stains, such as for AD beta amyloid plaques and neurofibrillary degeneration, this pipeline will be useful in the development of neuropathologic atlases as well.

Disclosures: E. Turschak: None. **P. Balaram:** None. **K. Takasaki:** None. **N. Postupna:** None. **E. Peterson:** None. **A. Edgren:** None. **N. Ouellette:** None. **B. MacLennan:** None. **M. Taormina:** None. **A. Glaser:** None. **J. Waters:** None. **C.D. Keene:** None. **R.C. Reid:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.07

Topic: I.03. Anatomical Methods

Support: Canadian Institutes of Health Research (CIHR) grant FDN-143212 to M. Petrides
Natural Sciences and Engineering Research Council of Canada (NSERC) to K. Drudik

Title: Morphological patterns of the superior parietal sulcus in the human brain

Authors: *K. DRUDIK[1,2], V. ZLATKINA[1], M. PETRIDES[1,2];
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Abstract: The superior parietal sulcus (*sps*) is the main sulcus of the superior parietal lobule. The sulcal maps of the 20th century show the sps as a single and continuous sulcus that separates the anterior from the posterior portion of the superior parietal lobule (Economo, Koskinas 1925, *J. Springer*). The present study examined the morphology of this sulcus to provide criteria for its identification and its variants, and thus facilitate anatomo-functional correlations in functional neuroimaging studies. MRI scans were randomly selected from the International Consortium for Brain Mapping (Mazziotta et al. 2001, *Phil. Trans. R. Soc.*). These subjects had no history of neurological and/or psychiatric illness, and gave informed consent. Identification of the sps was based on criteria established in recent atlases of the sulcal morphology of the human cerebral cortex in MNI stereotaxic space (Petrides 2018, *Elsevier Academic*). Voxels of the sps were manually identified on individual *in-vivo* MRI brain volumes using an interactive imaging software package called Display (MacDonald 1996, *McConnell BIC*). The sps was examined in the coronal, horizontal, and sagittal sections at 1mm intervals to determine its direction, extent, and pattern with respect to neighboring sulci. The sps was identified in all hemispheres and the morphological patterns of the sulcus were classified into three groups. 1) In the majority of cases, the sps was a distinct sulcus that did not merge with other sulci, i.e. it was clearly separate from surrounding parietal sulci visible on the surface of the brain or by way of a submerged gyral bridge. 2) The sps merged with another parietal sulcus, such as the anterior ramus of the horizontal part of the intraparietal sulcus (*IPSa*), the superior postcentral sulcus (*spcs*), and less frequently the posterior ramus of the horizontal part of the intraparietal sulcus (*IPSp*). Finally, 3) the sps was observed in two or more branches that were distinct and running in proximity, or connected to form an sps complex. Less frequently, the sps complex was found as a group of dimples, rather than well-defined sulci. The present study examined the morphology of the

superior parietal sulcus and established consistent morphological patterns in a common anatomical space to facilitate structural and functional analyses of the superior parietal lobule.

Disclosures: K. Drudik: None. **V. Zlatkina:** None. **M. Petrides:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.08

Topic: I.03. Anatomical Methods

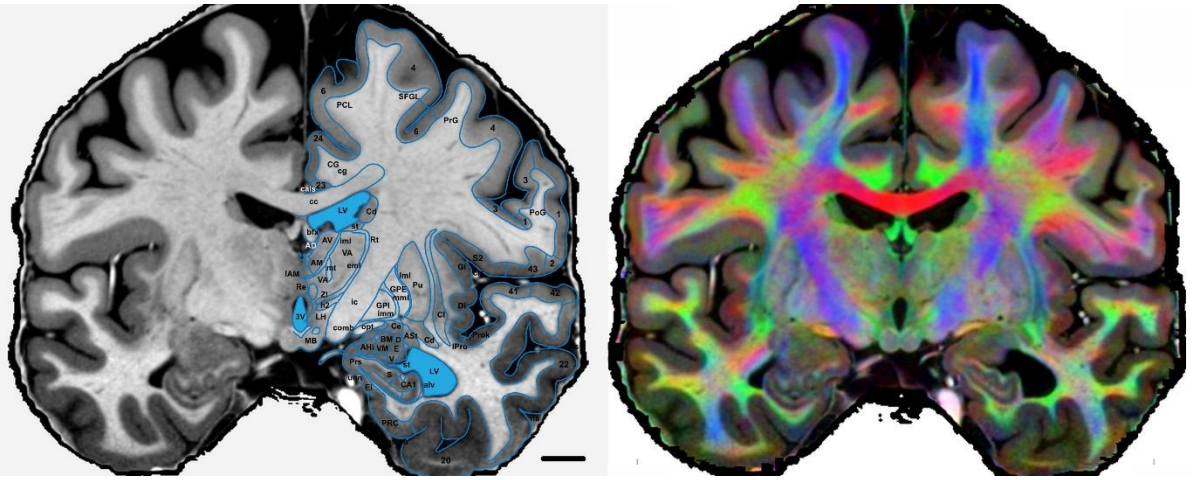
Support: NHMRC APP1140295

Title: Caress the Detail: A Human Brain in vivo MRI Reference Dataset.

Authors: *M. M. SCHIRA[1,3], G. PAXINOS[3], Z. J. ISHERWOOD[4,2], S. KASSEM[3], M. BARTH[5], T. B. SHAW[5], M. ROBERTS[6,1];
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Abstract: Virtually all major discoveries in neuroscience for the last 100 years have been underpinned by an increasingly detailed understanding of the architecture and connectivity. Brain atlases assist students, scientists and clinicians to navigate the brain and understand its function. Histological atlases, provide exquisite detail, accuracy and a long history of refinements and cross species references, but rely on invasive post mortem procedures and often distorted 2D slices. MRI atlases are 3D, rely on and inform in vivo applications, but are of significantly lower detail and annotations.
Introducing “Caress the Detail” an initiative aiming to provide a new generation of high-resolution, open-access brain atlases combining histology-derived annotation detail of human brain anatomy with cutting edge 3D in vivo MRI imaging. We present the first step of this initiative: a comprehensive dataset of two healthy male volunteers reconstructed at 0.25 mm isotropic spatial resolution for T1w, T2w and DWI contrasts. Multiple acquisitions for each contrast and each participant (20 T1w, 12 T2w, 10 DWI) and were averaged within subject using symmetric group-wise normalisation (Advanced Normalisation Tools). T1w and T2w data were acquired using a 7T human research scanner (Siemens MAGNETOM) at the Centre for Advanced Imaging, University of Queensland. T1w scans were recorded using a MP2RAGE sequence (WIP944) at 0.4mm3 isotropic resolution using: TR=4300ms, TE=1.8ms, TI1=830ms, TI2380ms, GRAPPA=2. T2w scans were recorded using a TSE sequence (WIP692) at 0.4 mm3 isotropic resolution using TR=1330ms, TE=118ms, GRAPPA 3. DWI scans were recorded with

a 3T MRI (Philips Achieva CX) (NeuRA Imaging Centre) using an inverse blip corrected SPIR sequence at 1.25 mm3 isotropic resolution, with 32 directions, 5 b-factor averages, B-val=1000, TE=60ms, TR=26.5s, SENSE=3. The high-resolution datasets, the analysis protocols and the annotations will be made available via the platform hba.neura.edu.au, inviting the community to enjoy the detail and contribute annotations and analysis.



Disclosures: M.M. Schira: None. **G. Paxinos:** None. **Z.J. Isherwood:** None. **S. Kassem:** None. **M. Barth:** None. **T.B. Shaw:** None. **M. Roberts:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.09

Topic: I.03. Anatomical Methods

Support: Pilot Project Grant Quebec Bioimaging Network

Title: Longitudinal changes in gray to white matter T1-weighted contrast and T2* relaxation times in an ex vivo-in situ human brain fixed by perfusion

Authors: E.-M. FRIGON[1], I. R. LEPPERT[2], C. L. TARDIF[3], M. DADAR[4], ***J. MARANZANO**[5];
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Abstract: Understanding MRI changes of post-mortem human brain during fixation is key to the comprehension of ex vivo MRI-histology studies. Changes in MRI contrast and relaxation times reflect changes in tissue properties. Those changes are progressive since the fixation period may extend for several weeks or months. Changes in relaxation times have been previously described in brains fixed by immersion, showing progressive shortening, reaching a plateau at 6 months (Dawe RJ et al. Magn Reson Med. 2009). Differences in T2 relaxation times between the superficial and deep brain tissue have also been noted in immersed-fixed brains (Raman MR et al. Magn Reson Med. 2017).To our knowledge, changes in T2* relaxation times and gray matter (GM) to white matter (WM) T1-weighted (T1w) contrast in in situ, perfused-fixed brains have not been reported during the length of the fixation period. We used a human brain fixed in situ by whole body perfusion of a 10% formalin solution through the carotid artery (anatomists' technique). We subsequently severed the head and proceeded to acquire monthly scans for six months. We collected T1w (MPRAGE), T2weighted (T2w), and multi-echo gradient (GRE) sequences. We used T1w and T2w contrasts to generate GM and WM, masks. We segmented areas showing high T1w GM-WM contrast, and areas with low T1w GM-WM contrast. qMRlab (MATLAB based open-source software for quantitative MRI analysis) was used to map the T2* relaxation times curves from the multi-echo GRE images.The volume of brain tissue showing a high T1w GM-WM contrast progressively decreased over time, while the volume showing low T1w GM-WM contrast progressively and reciprocally increased over time. We also detected progressive shortening of T2* values with time. Interestingly, the tissue experiencing loss of GM-WM contrast at each visit showed different levels of T2* shortening, with tissues losing GM-WM contrast at first visit showing the shortest T2* times (GM 45.9 ms ±1.2; WM 33.4 ms ±0.9), tissue losing GM-WM contrast later (3rdvisit) showing the longest T2* times (GM 50.1 ±0.9; WM 37.6 ms ±1.2), and tissue losing contrast even later (5th visit) showing times that shorten again (GM 48.6 ±0.5; WM 35.0 ms ±0.7).These results show that the time elapsed from the loss of GM-WM contrast on T1w is related to the magnitude of the shortening in T2* relaxation times, potentially reflecting different degrees of fixation in these areas and/or some degree of pre-fixation changes (decomposition) before fixation takes place.

Disclosures: E. Frigon: None. **I.R. Leppert:** None. **C.L. Tardif:** None. **M. Dadar:** None. **J. Maranzano:** None.

Poster

P922. Sample Preparation and Novel Probes

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P922.10

Topic: I.03. Anatomical Methods

Support: P50 AG005136
P30 AG00665

T32 AG052354
the Nancy and Buster Alvord Endowment

Title: Leveraging neuroimaging tools to assess precision and accuracy in an alzheimer's disease neuropathological sampling protocol

Authors: *J. WEBSTER[1], T. MADHYASTHA[1], L. GIBBONS[2], C. D. KEENE[3], T. J. GRABOWSKI, Jr[4], C. S. LATIMER[3];
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[4]Radiology, Univ. of Washington Dept. of Radiology, Seattle, WA

Abstract: INTRODUCTION: The study of Alzheimer's disease (AD) investigates topographic patterns of degeneration in the context of connected networks of functionally distinct domains using increasingly sophisticated molecular techniques. Therefore, obtaining high precision and accuracy of neuropathologic tissue sampling will enhance the reliability of molecular studies and contribute the understanding of Alzheimer's pathology. Neuroimaging tools can help assess these aspects of current sampling protocols as well as contribute directly to their improvement. **METHODS:** Using a virtual sampling method on magnetic resonance images from 35 participants (ages 59-91; 21 women), we compared the precision and accuracy of traditional neuropathological vs. neuroimaging-guided sampling. The impact of the resulting differences was assessed by evaluating the functional connectivity pattern of regions selected by each approach. **RESULTS:** Virtual sampling using the traditional neuropathologic approach had low neuroanatomical accuracy and precision for all cortical regions tested. Neuroimaging-guided strategies narrowed these gaps. Discrepancies in the location of traditional and neuroimaging-guided samples corresponded to differences in functional connectivity.**DISCUSSION:** Integrating neuroimaging tools with the neuropathologic assessment will improve neuropathologic-neuroimaging correlations, which is particularly important for linking antemortem clinical assessments and neuroimaging data to postmortem cellular and biomolecular findings. Our neuroimaging-based simulation of current sampling practices provides a benchmark of accuracy and precision against which to measure improvements with novel tissue sampling approaches. Our results suggest that relying on gross landmarks alone to select samples at autopsy leads to significant variability, even when sampled by the same neuropathologist. Further, this exercise highlights how sampling precision could be enhanced if neuroimaging were integrated with the standard neuropathologic assessment. More accurate targeting and improved biological homogeneity of sampled brain tissue will facilitate the interpretation of neuropathological analyses in AD and the downstream research applications of neural tissue from biorepositories.

Disclosures: J. Webster: None. **T. Madhyastha:** None. **L. Gibbons:** None. **C.D. Keene:** None. **T.J. Grabowski:** None. **C.S. Latimer:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.01

Topic: I.04. Physiological Methods

Support: NIH R01NS099429
NIH R01NS078223
T32EB014855

Title: Simultaneous Functional and Thermal Imaging of Mouse Cortex

Authors: *S. CHEN[1], H. BANKS[2], J. BUMSTEAD[1], L. BRIER[2], A. BICE[2], S. GAINES[2], J. CULVER[2];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Wide-field two photon microscopy is able to map mouse brain functions with large field of view (FOV) at cellular resolution. However, due to the decrease of pixel dwell time, obtaining sufficient signal to noise ratio (SNR) at high frame rate remains a challenge. Although increasing laser power can potentially improve SNR, a technique for monitoring the mouse brain temperature under high laser power is needed to ensure the mouse will be imaged safely. In this study, we proposed an imaging setup for simultaneous functional and thermal imaging for monitoring and studying the thermal effect of laser light on mouse cortex during the scan. This was achieved by using an air-coupled, long working distance objective with an Indium-tin-oxide slide placed on top of the mouse cranial window. The infrared light was reflected by the slide and redirected to the thermal camera. We found that the Indium-tin-oxide slide has around 85% transmission of 900nm light, and the image quality did not degrade significantly. Using this setup, we monitored the brain as a function of scan area and laser power. As anticipated, for a fixed laser power, the max temperature of the brain decreases as the area of the laser scanning increases, demonstrating the possibility of using higher laser power when scanning larger areas. With a large 5 x 5 mm FOV, we found that the mouse brain peak temperature at the surface was 39°C during a scan with 400mW laser power, whereas for a smaller 1 x 1 mm FOV the peak temperature was 39°C for only 200 mW of laser power. We confirmed that two photon signal scales as the power squared, so that the large 5 x 5 mm FOV had 4x more signal with 400 mW than it did with 200 mW. On the other hand, the large FOV also had shorter dwell times. Keeping the same frame rate and number of lines, the dwell time per equivalent pixel size is 5 times smaller for 5 x 5 mm FOV vs 1 x 1 mm FOV. This is approximately balanced out by the gains in signal increase from the higher power. We will present the full parameter space results of FOV and power, and also the results of feasibility studies evaluating brain activities both under hind paw stimulation and at rest. Together, these results demonstrated that high laser power for wide-field two-photon imaging can potentially be used for obtaining sufficient SNR functional data with little damage to the mouse cortex.

Disclosures: S. Chen: None. **H. Banks:** None. **J. Bumstead:** None. **L. Brier:** None. **A. Bice:** None. **S. Gaines:** None. **J. Culver:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.02

Topic: I.04. Physiological Methods

Support: NIH Grant 5R01DC014487-05

Title: Axially independent optical control and readout of neuronal circuit activity using the olfactory bulb as a model.

Authors: D. F. ALBEANU[1], M. S. KOH[1], F. ANSELMI[1], S. SADHU[1], P. GUPTA[1], H. CHAE[1], D. HERNANDEZ-TREJO[1], P. S. VILLAR[2], *A. BANERJEE[1];
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Abstract: All-optical methods for manipulating and measuring neuronal activity in vivo is a promising strategy to map the functional connectivity of layer-organized neural circuits at cellular resolution. We manipulated neural activity via one-photon patterned illumination with optogenetic stimulation and coupled this with quasi-simultaneous optical readout of neuronal activity using multiphoton microscopy. We used a digital micro-mirror device (DMD) to create patterned illumination profiles. Axial separation of stimulation and readout planes (up to 500 µm axial shift), was implemented by conjugating a motorized translatable holographic diffuser with the desired photo-stimulation plane, thus achieving widefield axial optical sectioning (15 µm x-y, 30 µm z-resolution, across 1.5 x 1.5 mm). To demonstrate the utility of stimulating and monitoring neural activity at two independent optical planes, we specifically controlled the activity of single glomeruli (stimulation of OSN terminals in OMP-Cre x ReaChR mice) on the surface of the olfactory bulb, while monitoring responses (GCaMP6f/s/7b) of the output neurons (mitral and tufted cells). We sampled mitral and tufted cell responses either within the photo-stimulation plane (glomerular dendritic tufts) or at 150-250 µm (cell bodies) below the photo-stimulation plane across a range of photo-stimulation intensities. Glomerular stimulation of OSN terminals selectively evoked responses in the targeted glomerulus and in the cell bodies of sister mitral and tufted cells receiving input from the photo-stimulated glomerulus and, further, revealed lateral inhibitory interactions between glomeruli. In ongoing experiments, we analyze the odor response properties of the identified sister cells as a function of brain state and experience. This technique can be readily applied to other brain regions that have layer-specific organization such as the neocortex.

Disclosures: D.F. Albeanu: None. **M.S. Koh:** None. **F. Anselmi:** None. **S. Sadhu:** None. **P. Gupta:** None. **H. Chae:** None. **D. Hernandez-Trejo:** None. **P.S. Villar:** None. **A. Banerjee:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.03

Topic: I.04. Physiological Methods

Support: NSF CBET 1631912
NSF BIO 1926676
NIH UF1 NS116241

Title: Further development of the GRINtrode, a head-mounted miniature microscope with simultaneous extracellular electrophysiology, and analysis of optical vs. electrical data

Authors: *C. MCCULLOUGH, T. CORRIGAN, D. RAMIREZ-GORDILLO, G. FUTIA, M. YOUNG, E. GIBSON, D. RESTREPO;
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Abstract: *In-vivo* imaging of neural activity has become a powerful tool for researching neural networks in action. The most popular optical reporter, GCaMP, is a calcium-based fluorescent reporter that provides excellent signal-to-noise ratio, is available in transgenic mouse lines, and is compatible with a wide variety of imaging methods. However, GCaMP is inherently limited in temporal resolution due to its dependence on calcium dynamics. This limitation makes it difficult to identify individual action potentials in optical data collected using GCaMP. *In-vivo* extracellular electrophysiology is a direct measure of neural activity that allows researchers to identify single action potentials as well as record the local field potential. To allow researchers the benefits of both recording modalities, we have developed a GRIN lens-based head-mounted miniature microscope, the GRINtrode, that provides simultaneous extracellular electrophysiology with 4 tetrodes on the periphery of the GRIN lens. Here we present our progress with development of the GRINtrode, including demonstration of fiber-coupling capability, and analysis of the relationship between GCaMP and tetrode data.

Disclosures: C. McCullough: None. **T. Corrigan:** None. **D. Ramirez-Gordillo:** None. **G. Futia:** None. **M. Young:** None. **E. Gibson:** None. **D. Restrepo:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.04

Topic: I.04. Physiological Methods

Support: UF1 NS107705
U01 NS103517

Title: Fast two-photon voltage imaging with photo-stable, genetically-encoded indicators

Authors: *J. PLATISA[1,2], P. F. O'BRIEN[1], R. F. O'BRIEN[1], L. A. DELGADO[1], J. O. PETTY[1], X. YE[4,5], A. AHRENS[6], J. L. CHEN[4,5,6], V. A. PIERIBONE[1,2,3];
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Abstract: Two-photon voltage imaging with genetically encoded indicators is a non-invasive, reliable method to record membrane electrical transients from deeper brain structures with cellular resolution. However, the lack of high-performing 2P-sensitive indicators and rapid imaging systems significantly limits the application of this approach. The best opsin-based GEVIs are sensitive and fast enough to report individual action potentials in collections of superficial neurons under single-photon excitation. However, this class of GEVIs is voltage insensitive under multi-photon excitation. High-speed, multi-photon voltage imaging has been demonstrated using ASAP3, the VSD-based GEVI. However, optical properties of ASAP3 (i.e., brightness and bleach rate) will need to be optimized to facilitate long-lasting in vivo imaging sessions in the mouse brain. Additionally, brighter and more photo-stable VSD-based variants (e.g., ArcLight-family) have slower kinetics that reduces spike detection fidelity. To develop GEVI variants optimized for multi-photon imaging, we have developed a semi-automated, multidimensional 2P-based screening platform. This unique system supports fast (~2hours/96-well plate) multidimensional screening of relevant GEVI features (brightness, photo-stability, and voltage sensitivity) in hundreds of cells simultaneously. The structure-guided protein evolution supported by this high throughput screening system resulted in several ultra-fast, sensitive, and photo-stable GEVIs optimized for prolonged multi-photon imaging.

Disclosures: J. Platisa: None. **P.F. O'Brien:** None. **R.F. O'Brien:** None. **L.A. Delgado:** None. **J.O. Petty:** None. **X. Ye:** None. **A. Ahrens:** None. **J.L. Chen:** None. **V.A. Pieribone:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.05

Topic: I.04. Physiological Methods

Support: U01 NS103517
N6600117C4012
N6600119C4020

Title: Dual polarity voltage imaging reveals state-dependent spiking and subthreshold dynamics in multiple cell types

Authors: *G. VASAN[1], M. KANNAN[2], S. HAZIZA[4], C. HUANG[5], J. LUO[6], M. J. SCHNITZER[7], V. A. PIERIBONE[3];
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Abstract: Genetically encoded voltage indicators have the potential to uncover millisecond-scale interactions between multiple identified cell types in live animals. However, current indicators have reduced voltage sensitivities and are incompatible for simultaneous multipopulation imaging. Here, we report next-generation FRET-opsin indicators Ace-mNeon2 and VARNAM2 with improved sensitivities and their respective reverse response-polarity variants pAce and pAceR. Combining the dual-color and dual-polarity indicators, we capture the simultaneous spiking and subthreshold dynamics of large ensembles belonging to up to three identified excitatory and inhibitory subpopulations in awake mice and flies. We further unravel inter-population voltage cross-correlations during behavioral state transitions. By overcoming the limitation of spectral bandwidth, and with enhanced signal-to-noise ratios, our dual-polarity indicators provide the most comprehensive insight to-date into cellular resolution voltage dynamics across identified subpopulations.

Disclosures: G. Vasan: None. **M. Kannan:** None. **S. Haziza:** None. **C. Huang:** None. **J. Luo:** None. **M.J. Schnitzer:** None. **V.A. Pieribone:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.06

Topic: I.04. Physiological Methods

Support: NIH Grant R01CA213149
NIH Grant R01EB023232
NIH Grant R01CA241618

AFOSR Grant FA9550-17-1-0387

Title: Label-free optical microscopy of millisecond-scale neural activity using phase-sensitive and polarization-sensitive full-field interferometry in optically sensitive neurons

Authors: *R. R. IYER[1,2], Y.-Z. LIU[1], C. A. RENTERIA[1,3], B. E. TIBBLE[1], H. CHOI[1], M. ŽURAUSKAS[1], S. A. BOPPART[1,2,3,4,5];
[1]Beckman Inst. of Advanced Sci. and Technol., [2]Dept. of Electrical and Computer Engin., [3]Dept. of Bioengineering, [4]Carle Illinois Col. of Med., [5]Cancer Ctr. at Illinois, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: We present a new versatile all-optical technique for neurophysiology that utilizes a novel label-free optical microscopy system called Superfast Polarization-sensitive Off-axis Full-field Optical Coherence Microscopy (SPoOF OCM). SPoOF OCM captures the changes to both the optical path length and birefringence induced by the millisecond-scale electrical activity of neurons. This multimodal response is imaged in a widefield configuration at 4000 frames per second, with a field-of-view of 200×200 μm^2, 1 μm transverse resolution, and 4.5 μm axial resolution. With an ability to capture responses spanning three orders of magnitude in both space and time, SPoOF OCM meets the exacting needs of a comprehensive neurophysiology tool. Hence, SPoOF OCM overcomes the existing limitations of traditional electrophysiology and fluorescence microscopy. SPoOF OCM is validated in mammalian neurons by comparing the nanometer-scale optical path differences and qualitative birefringence of the scattered fields to field-potential electrophysiology measurements. The off-axis configuration, dual reference arms, and no moving parts ensure phase stability as low as 0.3 nm. The millisecond-scale responses for both spontaneous and electrically stimulated activity of mammalian neurons from every pixel in the field of view are used to estimate instantaneous connectivity patterns and videos of dynamic neural activity within neural circuits. We demonstrate an all-optical configuration of this system for excitation and stimulation of mammalian neurons. Differentiated mouse neuroectodermal stem cells (NE-4C) are transfected with channelrhodopsin-2 and its variant CHETA. The cells are excited, both locally and widefield, with different pulse duration and reputation rates. The responses to optical stimulation were imaged using SPoOF OCM to study the dynamics of the neural network and the interplay between the optically sensitive and wildtype cells in the co-culture. We highlight the noninvasiveness and versatility of an all-optical system and the higher data throughput of SPoOF OCM that overcomes the limitations of electrophysiology. Our label-free imaging setup is particularly relevant for research involving the retina and vision. Since SPoOF OCM can track the millisecond-scale activity of neurons across an entire field of view, it provides a framework to progress from low-throughput electrophysiology to highly parallelized, non-invasive, and label-free optophysiology.

Disclosures: R.R. Iyer: None. **Y. Liu:** None. **C.A. Renteria:** None. **B.E. Tibble:** None. **H. Choi:** None. **M. Žurauskas:** None. **S.A. Boppart:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.07

Topic: I.04. Physiological Methods

Support: NIH UF1 NS107705

Title: Multi-beam ultra-fast two-photon microscope for population-level voltage imaging in mouse cortex

Authors: *X. YE[1,2], A. M. AHRENS[3], N. MANJREKAR[3], J. C. M. PLATISA[5,6], V. A. PIERIBONE[5,6,7], H. WANG[4], L. TIAN[4,1], J. L. CHEN[3,1,2];
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Abstract: Understanding the complexity of neural networks *in vivo* requires simultaneous recordings of neuronal populations with action potential resolution. Genetically-encoded voltage indicators (GEVIs) provide direct and reliable optical readouts of neural activity with high temporal resolution. The fast dynamic of spike-evoked GEVI responses necessitates the development of kilohertz-rate two-photon (2P) imaging systems. Existing high-speed 2P microscopes scan restricted field of views (FOVs), limiting the number of simultaneously imaged neurons. We developed an Ultra-Fast 2-Photon (UF2P) microscope which is capable of full-frame high-speed imaging of a 400x400μm^2 FOV of up to ~300 microns deep in mouse cortex at a kilohertz scan rate. We used a combination of spatial and temporal beam multiplexing to achieve parallel resonance frame scanning of 8 beams at up to 1 kHz framerate. Spatially multiplexed beams were spaced 200μm apart to avoid crosstalk from light scatter in deeper tissue depths and emitted photons were resolved using a multi-anode photomultiplier tube. For temporal demultiplexing, a fixed 920nm-wavelength, low repetition rate laser was used to deliver interleaved laser pulses and emitted photons were digitized and temporally de-multiplexed with custom electronic components. We compared the photobleaching performance and neural activity readouts of two-photon compatible GEVIs in the primary somatosensory cortex of awake mice. Using a self-supervised deep-learning-based denoising algorithm, sensory-evoked action potentials could be identified from neurons imaged at resolutions near 1μm^2/pixel. An automated in vivo patch clamp system was integrated into the microscope to enable simultaneous electrophysiology recordings to potentially characterize GEVIs using the system. This microscope system demonstrates the capacity to perform two-photon voltage imaging across larger neuronal populations.

Disclosures: X. Ye: None. **A.M. Ahrens:** None. **N. Manjrekar:** None. **J.C.M. Platisa:** None. **V.A. Pieribone:** None. **H. Wang:** None. **L. Tian:** None. **J.L. Chen:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.08

Topic: I.04. Physiological Methods

Support: McKnight Foundation

Title: Ultra-lightweight and large field-of-view head-mounted microscopes for in vivo neural recording

Authors: *J. SCHERRER, G. F. LYNCH, J. ZHANG, M. S. FEE;
Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Miniature head-mounted microscopes have enabled in vivo optical measurements of neural activity in freely moving animals. Recent improvements to these techniques have both decreased device weight and increased field-of-view (FOV). Light-weight devices enable neural recordings in freely behaving small animals, while increased FOV enhances the number of neurons that can be simultaneously observed. However, existing microscope designs suffer from a fundamental tradeoff between weight, FOV, and resolution. Microscopes optimized for low weight suffer from low resolution and FOV, while conversely designs optimized for a large FOV (greater than 1 mm^2) are too heavy to be used in small animals such as songbirds.
Here, we present two different head-mounted microscopes that overcome these limitations by using a novel compact optical design. Specifically, we eliminate the need for a dichroic filter by instead introducing excitation light at the back aperture of the objective lens with an optical fiber coupled to a small prism. The first design (Featherscope) weighs just 1.0 g while maintaining cellular resolution across 1.0 x 1.0 mm FOV, enabling neural recordings in animals that are too small for existing head-mounted microscopes, such as juvenile songbirds. The second design (wide FOV scope) provides cellular resolution imaging across 4.8 x 3.6 mm FOV in a device weighing only 1.4 g, opening the door to wide-field neural recordings in freely behaving mice and songbirds. We have used these microscopes to collect the first calcium recordings of LMAN in juvenile zebra finches during subsong. We have also obtained simultaneous recordings from several hundred neurons in multiple cortical of a freely-behaving mouse.

Disclosures: J. Scherrer: None. **G.F. Lynch:** None. **J. Zhang:** None. **M.S. Fee:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.09

Topic: I.04. Physiological Methods

Support: NIH EY028381

Title: A coded exposure camera to enhance the speed and SNR of GEVI imaging

Authors: *J. ZHANG[1], J. P. NEWMAN[1], G. BROWN[4], C. LINGHU[2], P. CHIN[4], E. S. BOYDEN[2], M. A. WILSON[3];
[1]Dept. of Brain and Cognitive Sci., [2]Media Lab., [3]Picower Inst. Learn/Memory, MIT, Cambridge, MA; [4]Computer Sci., Boston Univ., Boston, MA

Abstract: Genetically encoded voltage indicators (GEVIs) permit cell-type-specific measurements of spiking activity and subthreshold voltages. However, the speed requirement for wide-field, spike-resolution GEVI imaging presents significant challenges for conventional image sensors: fast frame rates (>1 kHz) lead to short photon integration times (<1 ms) of already weak fluorescent signals. On the other hand, GEVI videos have significant spatiotemporal correlations that can be exploited to improve SNR while maintaining temporal resolution using non-traditional sampling architectures. To this end, we designed a digital camera that allows independent exposure control over every pixel. We show that by temporally staggering pixel exposures (“coded staggered exposure”; CSE), we can accurately capture GEVI signals free of aliasing even with sub-Nyquist, per-pixel sampling rates. Using the CSE image sensor, we imaged and resolved 1KHz-equivalent membrane voltage activity using a sampling rate < 200 Hz and pixel integration time > 5 ms. Further, we show that CSE outperforms global pixel exposure used in conventional CMOS cameras, especially at higher noise levels typically seen in miniature, head-mounted microscopes that are not actively cooled. Our CSE image sensor is implemented using a 180nm CMOS image sensor process and contains 240 x 352 pixels with a 7um pixel pitch occupying an area of 5 x 4 mm. The CSE image sensor is fully integrated and achieves pixel exposure staggering without, for instance, external optical modulation. Thus it can be used directly in place of a standard image sensor in both head-fixed and freely moving animal experiments.

Disclosures: J. Zhang: None. **J.P. Newman:** None. **G. Brown:** None. **C. Linghu:** None. **P. Chin:** None. **E.S. Boyden:** None. **M.A. Wilson:** None.

Poster

P929. Optical Methodology: Development

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P929.10

Topic: I.04. Physiological Methods

Support: NS115585

Title: Www.3photon.org provides information about 3-photon excitation.

Authors: *J. WATERS;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Much like 2-photon microscopy, 3-photon microscopy uses IR pulsed laser light to drive multiphoton excitation. 3-photon excitation extends the maximum imaging depth towards 2 mm, considerably deeper than is possible with 2-photon excitation. We explored the depth limit of 2-photon microscopy, building a microscope that performed near-simultaneous 2- and 3-photon excitation and thereby enabling a direct comparison of 2- and 3-photon images. Two-photon and three-photon results from superficial locations were almost identical, with the tuning properties from neurons in V1 being indistinguishable. Two-photon results were inaccurate beyond ~450 um in mice with dense GCaMP expression, shallower than predicted by calculations. Information on our microscope design, and much more information about 3-photon microscopy, is available at www.3photon.org. One of the main purposes of www.3photon.org is the provision of information on the key differences between 2- and 3-photon excitation, to facilitate adoption of 3-photon excitation. 3photon.org pulls together information from the literature about - the properties and limits of 3-photon excitation - fluorophore cross-sections - microscope components - pulse shape, dispersion and dispersion compensation - data sets and software tools - publications - the latest advances Our results and the information available at www.3photon.org compare the performance of 2- and 3-photon excitation *in vivo* and provide information to facilitate the adoption of 3-photon excitation.

Disclosures: J. Waters: None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.01

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

Support: NIH Grant 1OT2OD030534 (SPARC Program)

Title: SPARC: computational modeling of right atrial ganglionic plexus (RAGP) neurons from transcriptomics to electrophysiology

Authors: *J. FELDMAN[1], **S. GUPTA**[2], L. KUTTIPPURATHU[3], A. MOSS[3], J. S. SCHWABER[3], R. VADIGEPALLI[3], W. W. LYTTON[2,4];
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Genomics/Computational Biology, Thomas Jefferson Univ., Philadelphia, PA; [4]Neurol., Kings County Hosp., Brooklyn, NY

Abstract: Neurons in the right atrial ganglionic plexus (RAGP), a component of the intrinsic cardiac nervous system, mediate vagal modulation of the SA node. RAGP neuron anatomical, electrophysiologic, and molecular properties have been studied in vitro in rat and mini pig. We used transcriptomic data to inform single-compartment computational models of RAGP neurons. We created neuronal models of phenotypically distinct neurons from genes coding for ion channels in the RAGP as identified in a transcriptomic map generated from qPCR and RNAseq for 405 single RAGP principal neurons in mini pig. We mined the transcriptomic data to identify ion channels encoded by expressed genes and evaluated kinetic models using Hodgkin-Huxley formalisms available on Ion Channel Genealogy, Channelpedia, and ModelDB. From the transcriptomics, we identified 115 unique cell types defined by different combinations of 12 ion channels including Naf (*Scn1a*), KDR (*Kcnc1*, *Kcna1b1*), P-, L-, N-, and T-type Ca^{2+} (*Cacna1a*, *b*, *c*, *g*, *i*), and *HCN1*, *HCN2*, *HCN3*, and *HCN4*. Conductance values in the RAGP neuronal models were optimized to generate realistic firing responses to a current clamp. Across 3 parameter sets and a range of current input values, 5 cell responses were observed: subthreshold, single-spike phasic, tonic, and tonic (i) during stimulus and (ii) continuing post-stimulus, consistent with published experimental data on RAGP neurons in rat. Pooling responses across parameter sets, at 0.5 nA 47.0% (162/345) were single-spike phasic, 27.5% (95/345) tonic with 0.6% (2/345) brief and 2.3% (8/345) sustained post-stimulus; at 0.8 nA 47.0% were phasic, 28.1% tonic with 0% brief and 2.3% sustained post-stimulus; and at 1.0 nA 47.0% again were phasic, 23.0% tonic with 3.5% brief, 3.5% sustained. Overall, we found that multiple transcriptomically distinct neuronal models generated a limited repertoire of cell responses. We next aim to simulate networks of these neuronal models, incorporating anatomic and neuromodulatory data collected earlier to help elucidate the role of RAGP in the pacemaker function of the heart.

Disclosures: S. Gupta: None. **L. Kuttippurathu:** None. **A. Moss:** None. **J.S. Schwaber:** None. **R. Vadigepalli:** None. **W.W. Lytton:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.02

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

Support: SPARC Program, NIH 3OT2OD028191-01S1
Advanced Research Computing at the University of Michigan, Ann Arbor

Title: Patient-specific computational modeling of pudendal nerve stimulation

Authors: *L. RUIZ PEREZ[1], E. MIRZAKHALILI[1], A. KOTKAR[1], R. LAPETINA[4], P.-J. CHEN[2], A. LAGUNAS[1], G. SHAH[3], P. GUPTA[5], T. M. BRUNS[1], S. F. LEMPKA[1]; [1]Dept. of Biomed. Engin., [2]Mol. Cell. and Developmental Biol., [3]Dept. of Radiology, Univ. of Michigan, Ann Arbor, MI; [4]Univ. of Puerto Rico Sch. of Med., San Juan, PR; [5]Dept. of Urology, Univ. of Michigan, Ann Arbor, MI

Abstract: Bladder dysfunction stemming from aging, trauma, surgery, or disorders of the nervous system impairs the quality-of-life and productivity of millions of individuals. Lifestyle changes and pharmaceuticals are often insufficient to resolve bladder dysfunction; therefore, neurostimulation-based approaches are of particular interest. The pudendal nerve, which forms from the S2-S4 spinal nerve roots and innervates the genitals, perineum, urethra, and anal sphincter, is the target of one such emerging neuromodulation technique that may ameliorate bladder dysfunction. Pudendal nerve stimulation has been examined extensively in preclinical studies. However, there have been few studies of direct nerve stimulation in patients and little is understood about variations in effectiveness and outcomes across patients. The present work aims to investigate pathway activation during pudendal nerve stimulation through the use of patient-specific computational models of human participants receiving pudendal nerve stimulators. Specifically, we developed volume conductor models of participants' pelvic anatomy by segmenting magnetic resonance and computed tomography images. We assigned physiologically appropriate electric conductivities to the various tissue domains of the volume conductor models and used the finite element method to predict the potential fields generated by the stimulation. To estimate the neural response to stimulation, we applied the potential fields to multi-compartment cable models of axons spatially arranged so as to approximate the nerve's course, branching, and fascicular topology. Our results characterized relative recruitment of the genital, perineal, and rectal fascicles of the pudendal nerve in the participant population during pudendal nerve stimulation. Furthermore, our models demonstrated recruitment differences for different stimulation configurations and pulse widths. In future work, we will utilize these models to establish theoretically optimal stimulation parameters that maximize excitation of targeted fascicles and minimize excitation of off-target fascicles. We believe that this patient-specific modeling approach will help increase the clinical efficacy of pudendal nerve stimulation for treating bladder dysfunction.

Disclosures: L. Ruiz Perez: None. **E. Mirzakhalili:** None. **A. Kotkar:** None. **R. Lapetina:** None. **P. Chen:** None. **A. Lagunas:** None. **G. Shah:** None. **P. Gupta:** None. **T.M. Bruns:** None. **S.F. Lempka:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hologram Consultants LLC, Presidio Medical Inc. F. Consulting Fees (e.g., advisory boards); Abbott Neuromodulation.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.03

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

Support: NIH SPARC Program OT2 025340

Title: Sparc- analysis of individual-specific computational models of vagus nerve stimulation (vns) in humans, pigs, and rats

Authors: E. D. MUSSELMAN, N. A. PELOT, W. M. GRILL;
Duke Univ., Durham, NC

Abstract: Preclinical studies show promise to expand use of VNS to treat a range of diseases and disorders beyond epilepsy and depression, but translation of therapies across species and to the clinic remains a challenge. Computational modeling of nerve responses to VNS is a valuable tool to facilitate translation of stimulation parameters by accounting for differences in nerve and electrode geometries. We used our ASCENT (Automated Simulations to Characterize Electrical Nerve Thresholds) pipeline to model stimulation of human (n=9), pig (n=12), and rat (n=9) cervical vagus nerves. ASCENT created a finite element model of each nerve, cuff electrode, and surrounding tissue in COMSOL to calculate the extracellular potentials. ASCENT simulated the responses of mammalian myelinated fibers (10 μm MRG) to electrical stimulation (100 μs/phase biphasic pulse) in NEURON by applying the time-varying extracellular potentials. We used linear regression and principal component analysis to determine how thresholds of activation related to features of nerve geometry across our population of VNS models.
Thresholds to activate model myelinated axons were ~4x higher in humans than in pigs, and humans had ~20% higher coefficient of variation (CV) than pigs. Thresholds in rats were ~23x and ~7x lower than in humans and pigs, respectively, and rats had ~5x lower CV than pigs. Fascicle size and distance from outer epineurium surface were positively correlated with threshold (R = 0.7 and 0.36, respectively), and fascicle number negatively correlated with threshold (R = -0.65). The first principal component accounted for ~36% of the variability in threshold and included large weights for terms corresponding to fascicle characteristics (i.e., number and size of fascicles, proportion of cross section occupied by perineurium), the second accounted for ~24% of the variability and included large weights for terms corresponding to proportion of nerve occupied by endoneurium and epineurium, and the third accounted for ~18% of the variability and included large weights for terms corresponding to nerve size and fascicle distance to the outer epineurium surface.
Our models highlight the utility of ASCENT to create models of VNS for individual-specific nerves in response to stimulation. We demonstrate an approach to model-based analysis that revealed how nerve characteristics affect fiber response to stimulation. Mean activation thresholds and their CV increase from rats to pigs to humans. A fundamental understanding of how individual-specific nerves respond to a set of stimulation parameters will enable innovations to target selectively fiber responses.

Disclosures: E.D. Musselman: None. **N.A. Pelot:** None. **W.M. Grill:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.04

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

Support: SPARC Program: NIH Grant 1OT20D030535-01

Title: Sparc: closed-loop optimization of vagus nerve stimulation for the cardiovascular system using long short-term memory neural network

Authors: A. BRANEN[1], Y. YAO[2], M. V. KOTHARE[2], B. MAHMOUDI[3], *G. KUMAR[4]; [1]Chem. and Biol. Engin., Univ. of Idaho, Moscow, ID; [2]Chem. and Biomolecular Engin., Lehigh Univ., Bethlehem, PA; [3]Emory Univ., Atlanta, GA; [4]Chem. and Materials Engin., San Jose State Univ., San Jose, CA

Abstract: Introduction: Cardiovascular diseases are the leading cause of mortality in the United States and remain a major health risk worldwide despite several pharmaceutical treatments, thus motivating a search for alternative therapies. Recently, vagus nerve stimulation (VNS) has been identified as a potential therapy for the treatment of cardiovascular diseases. A particular challenge preventing the application of VNS as treatment involves selecting the optimal stimulation parameters for a desired physiological response. A potential solution involves using physiological measurements as feedback to guide the VNS parameter selection in a closed-loop formulation.
Methods: In this computational work, we have developed an optimal closed-loop model-based control approach using a machine learning model that could be used to optimize the VNS parameter selection in a closed-loop sense. Specifically, we used a recently published biophysical computational model of a rat cardiac system to generate a synthetic input-output time-series dataset consisting of the VNS frequency and pulse width as the inputs and the heart rate and the mean arterial blood pressure as the outputs. Next, we trained a long short-term memory (LSTM) neural network using the synthetic data and used the trained model in a closed-loop model predictive control (MPC) framework to optimize the VNS parameters. The LSTM-based controller was used to control the heart rate and the mean arterial blood pressure of the full physiological system model. To provide additional context, we investigated the choice of the cost function, the controller design parameters, and the effect of model-mismatch on the overall closed-loop performance.
Results: Our simulation results show that the designed controller can drive the heart rate and the mean arterial blood pressure to the desired physiological set-points under various conditions. Additionally, our simulation results illustrate how various choices of the cost function in the closed-loop control formulation impact the selection and design of VNS parameters for the same

set-points.
Conclusions: We have developed a model-based closed-loop control approach using a data-driven machine learning model (LSTM) to optimize the VNS parameters for controlling cardiovascular physiology, thereby making the technique potentially suitable for use with real data.

Disclosures: A. Branen: None. **Y. Yao:** None. **M.V. Kothare:** None. **B. Mahmoudi:** None. **G. Kumar:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.05

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

Support: SPARC Program OT3OD025349
SPARC Program OT2OD023848
NHLBI grant U01HL133360
NHLBI grant R15 1R15HL137143-01A1
NINDS grant U01NS113867

Title: Sparc: a common spatial representation for the intrinsic cardiac nervous system

Authors: *M. OSANLOUY[1], R. CHRISTIE[1], C. LEUNG[2], S. ROBBINS[4], A. MOSS[4], M. HEAL[5], S. TAPPAN[5], R. VADIGEPALLI[4], J. CHEN[3], Z. CHENG[3], J. S. SCHWABER[6], D. NICKERSON[1], P. HUNTER[1];
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Abstract: SPARC: a common spatial representation for the intrinsic cardiac nervous system Mahyar Osanlouy[1], Richard Christie[1], ClaraLeung[2], ShainaRobbins[3], AlisonMoss[3], Maci Heal[4], SusanTappan[4], Rajanikanth Vadigepalli[3], JinChen[2], Zixi (Jack)Cheng[2], James S. Schwaber[3], David Nickerson[1], Peter Hunter[1]
[1]Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand[2]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA[3]Daniel Baugh Institute of Functional Genomics/Computational Biology, Department of Pathology, Anatomy, and Cell Biology, Thomas JeffersonUniversity, Philadelphia, PA, USA[4]MBF Bioscience, Williston, VT, USA
The intrinsic cardiac nervous system (ICNs)is a local, integrative neural circuitry system which mediates the vagal control of the heart. Accurate characterization of these neurons requires a

comprehensive quantification of their physical spatial distribution on the heart as well as their molecular and cellular properties (e.g., neuromodulator and neurotransmitter expression). We have developed an aspatial framework which allows for integrating various data on individual ICNwithinacommon3Dcoordinate system known as a scaffold. The scaffold is generated from a semantic description of connected, simple-shaped elements to follow the features of the heart tissue from different subjects. The general shape of the scaffold is algorithmically generated as an *in vivo* representation of the organ which can be mathematically morphed into subject-specific configurations. Data for this study include high-resolution image stacks from isolated rat and porcine right atria allowing comprehensive annotation and 3Dmapping of corresponding subsystems of ICN. Our scaffolds are able to accurately capture the spatial distribution of these data and provide a framework to compare and analyze the variability between subjects and species. Our framework canbe generalized for application to human heart and ICN, for accelerating development of neuromodulatory therapies totreat heartdisease.

Disclosures: M. Osanlouy: None. **R. Christie:** None. **C. Leung:** None. **S. Robbins:** None. **A. Moss:** None. **M. Heal:** None. **S. Tappan:** None. **R. Vadigepalli:** None. **J. Chen:** None. **Z. Cheng:** None. **J.S. Schwaber:** None. **D. Nickerson:** None. **P. Hunter:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.06

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

Support: Sparc Program 1OT2OD030535-01

Title: Sparc: control-core: a platform for closed-loop peripheral neuromodulation control systems

Authors: P. KATHIRAVELU[1], M. ARNOLD[2], P. SARIKHANI[1], S. AWASTHI[3], S. MURPHY[2], J. FLEISCHER[2], Y. YAO[2], M. KOTHARE[2], ***B. MAHMOUDI**[1];
[1]Emory Univ., Atlanta, GA; [2]Lehigh Univ., Bethlehem, PA; [3]Vellore Inst. of Technol., Vellore, India

Abstract: CONTROL-CORE is a multi-university project to develop a platform for designing closed-loop neurostimulation control systems to treat conditions affecting internal organs. It facilitates modular simulation and deployment of closed-loop peripheral neuromodulation control systems and is part of the larger SPARC project (DAT-, MAP-, and SIM-CORE). SIM-CORE has simulations on O2S2PARC of Physiological Models (PMs), which are abstractions of the behavior of organs under electrostimulation. CONTROL-CORE represents a fourth leg to the SPARC portfolio (closed-loop control algorithms for peripheral stimulation therapies) by

allowing the interaction between a controller and the PM, previously not feasible in SIM-CORE.The CONTROL-CORE protocol, known as concore, allows modular simulation of controller and PM nodes to run on different platforms, including O2S2PARC, local (Windows, macOS, or Ubuntu), Docker, and distributed implementations. Concore works with several languages (such as Python, Matlab, C++, and Verilog) so that the developers code in their preferred language(s). The local implementations allow developers to use concore to create simulations from source code with appropriate tools installed locally (e.g. Matlab license). The Docker implementation allows nodes to be containerized so that others may reuse them without manual installation, configuration, source code, or a Matlab license. The distributed implementation communicates via the internet through "wrappers" so that a controller can be local while the PM can be remote (e.g. running on O2S2PARC). Eventually, the controller will run on an embedded processor or custom circuit implanted in an animal experiment or a patient. Since the PM may be computationally intensive and/or may only be available on specific platforms (such as O2S2PARC), it would be desirable to test such a controller on a limited-resource machine while running the more computationally intensive PM on a different machine via an internet connection.We tested concore with cardiac PMs, including pulsatile and nonpulsatile rat models, and Model Predictive Control (MPC), Reinforcement Learning (RL), and Long-Short-Term Memory (LSTM) based controllers. The time to simulate one cardiac cycle in a local implementation is about 0.1 seconds, and in a distributed implementation, a couple of seconds (faster than O2S2PARC).

Disclosures: P. Kathiravelu: None. **M. Arnold:** None. **P. Sarikhani:** None. **S. Awasthi:** None. **S. Murphy:** None. **J. Fleischer:** None. **Y. Yao:** None. **M. Kothare:** None. **B. Mahmoudi:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.07

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

Support: SPARC

Title: Sparc: a closed-loop control approach to vagal nerve stimulation (vns) of the cardiac system.

Authors: *O. ADEODU[1], Y. YAO[2], M. KOTHARE[2];
[2]Chem. & Biol. Engin., [1]Lehigh Univ., Bethlehem, PA

Abstract: The lack of clarity in effective combinations of electrical stimulation parameters and inherent variability across patients have been put forward to explain the mixed outcomes from

clinical studies that explored vagal nerve stimulation (VNS) as an alternative treatment for a variety of cardiovascular diseases. Conceptually, a closed-loop formulation of VNS can resolve these issues. In this work, we report a computational study of the application of control theory to the design of optimal, closed-loop VNS regulation systems that automatically adjusts the amplitude and frequency of electrical stimulation, based on feedback measurements. We utilize a detailed model of the human cardiac system (Park et al. 2020)) that features pulsatile blood flow through the cardiovascular system and a multi-input, multi-output baroreflex system for the autonomous regulation of sympathetic and parasympathetic activity. To close the VNS control loop, we rely on the results of computational experiments that model the interaction of physiologically and externally induced action potentials within the vagal nerve. In addition, the distinct sensitivities of the afferent and efferent fibers within the vagal bundle to stimulation amplitude are also exploited in the design of the control algorithm. With suitable approximations to reduce computational complexity, nonlinear model predictive control (NMPC) is demonstrated through simulation as a potentially viable, closed-loop VNS control strategy for the short-term mitigation of the effects of cardiac pathology. Hence, this study represents a first step towards the design of a computational tool for the development of closed-loop personalized neuromodulation therapies.
Reference: Park James H., Gorky Jonathan, Ogunnaike Babatunde, Vadigepalli Rajanikanth, Schwaber James. 2020. Investigating the Effects of Brainstem Neuronal Adaptation on Cardiovascular Homeo-stasis. Frontiers in Neuroscience. 14:470

Disclosures: O. Adeodu: None. **Y. Yao:** None. **M. Kothare:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.08

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

Support: NIH OT2 grant 3OT2OD026580
NIH OT2 grant 1OT2OD026580
NIH NHLBI grant 2R01HL128537
NIH NHLBI grant 5R01HL128537
NIH NHLBI grant 5U01HL126273
NIH NHLBI grant 1R01HL152681
AHA career development award 19CDA34770101

Title: Sparc: atomistic modeling for a multi-scale predictive neurocardiac simulator

Authors: J. R. D. DAWSON[1,2], P.-C. YANG[2], K. R. DEMARCO[2], S. BEKKER[5], Y. HAN[2], K. C. ROUEN[1,2], K. NGO[1,2], T. J. LEWIS[3], V. YAROV-YAROVOY[2], C. E. CLANCY[2,4], ***I.**

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Abstract: Modulation of peripheral nerve activity is a promising tool for the treatment of multiple cardiovascular disorders such as arrhythmias, heart failure and hypertension. Multi-scale modeling of such neuromodulation allows to predict optimal disease treatment options and is an integral part of our collaborative multi-lab initiative funded by the NIH Common Fund program "Stimulating Peripheral Activity to Relieve Conditions" (SPARC). The goal of our project is to develop a predictive multi-scale neurocardiac simulator in order to understand autonomic control of the heart and reveal its fundamental mechanisms from atomic to organ system scale. At the molecular level, such neuromodulation by sympathetic and parasympathetic nervous systems is established at the neuromuscular junction by stimulating myocyte G protein-coupled receptor (GPCR) mediated signal transduction pathways with neurotransmitters like norepinephrine or acetylcholine. In this study we used atomistic structural modeling and molecular dynamics (MD) simulations to identify energetics and kinetics of neurotransmitter, G protein and β-arrestin interactions with key GPCRs at the neuroeffector junction, beta-1 and beta-2 adrenergic receptors (β_1AR and β_2AR) and type-2 muscarinic acetylcholine receptor (M_2R). We developed atomistic models of these proteins and their complexes in different conformational states using available structures and Rosetta structural modeling. These models were validated via molecular docking of βAR agonists norepinephrine and isoproterenol and MR agonists acetylcholine and carbachol followed by multi-microsecond-long all-atom MD simulations. State-dependent protein - ligand and protein - protein binding affinities and association / dissociation rates for those systems were estimated using enhanced sampling MD as well as implicit-solvent MM/PBSA and Brownian dynamics techniques. These quantities were used as parameters for our functional kinetic model of subcellular signaling in ventricular myocytes, which was able to predict rate of cyclic adenosine monophosphate (cAMP) production in good agreement with standard models and experiments. Furthermore, those models were integrated and used in cardiac tissue simulations, predicting effect of sympathetic and parasympathetic stimulation on heart rhythm in health and disease.

Disclosures: J.R.D. Dawson: None. **P. Yang:** None. **K.R. DeMarco:** None. **S. Bekker:** None. **Y. Han:** None. **K.C. Rouen:** None. **K. Ngo:** None. **T.J. Lewis:** None. **V. Yarov-Yarovoy:** None. **C.E. Clancy:** None. **I. Vorobyov:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.09

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

Support: Sparc Program OT2OD030535

Title: Sparc: adaptive closed-loop control of vagal nerve stimulation for regulating cardiovascular function using deep reinforcement learning: a computational study

Authors: *P. SARIKHANI[1], H.-L. HSU[2], M. ZEYDABADINEZHAD[1], Y. YAO[3], M. KOTHARE[3], B. MAHMOUDI[1];
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Abstract: Introduction: Vagal nerve stimulation (VNS) is actively being investigated for improving outcomes of multiple cardiovascular diseases by compensating for the dysfunction of the autonomic nervous system. Standard clinical approaches use exhaustive open-loop parameter search to determine proper stimulation parameters. However, open-loop testing is not efficient and there is a need for designing automated closed-loop control systems that continuously learn and adapt the VNS parameters to the underlying dynamics of the cardiovascular system, based on the physiological measures. We propose to leverage Reinforcement Learning (RL) techniques for designing adaptive VNS control systems that can learn from the interaction with the physiological systems. **Methods:** We used a pulsatile model of the integrated cardiovascular system and baroreflex regulation that predicted the nonlinear effects of VNS stimulation parameters (pulse width and frequency) in three locations on heart rate (HR) and mean arterial pressure (MAP) to design an RL-based VNS control system. Since the pulsatile model was computationally expensive, we used the pulsatile model to generate trajectories of HR and MAP values for 420000 randomly generated stimulation parameters and trained a temporal convolutional neural network (TCN) model as a reduced-order surrogate of the pulsatile model for computational efficiency. The mean squared error (MSE) of the TCN model on an unseen test set in single-step predictions was 0.000132 and the computations were 5.5 times faster than the pulsatile model. We designed a RL agent using the proximal policy optimization (PPO) algorithm to learn a generalized VNS control policy for regulating HR and MAP states based on closed-loop interaction with the TCN model in python. **Results:** The RL agent observed the current (HR-MAP) state at time t and took an action, i.e. delivering VNS with the parameters defined by the control policy. The TCN model returned the next state and a measure of reward at time step t+1 in response to the RL action. We used the squared exponential reward function for training the policy to simultaneously regulate HR and MAP to a desired set-point. The RL agent converged in 2000 episodes, where each episode included 500 cardiac cycles. We tested the performance of the RL agent in a set-point tracking task for three different set-points for HR and MAP for the duration of 100 cardiac cycles for each set-point. The RL agent achieved an average reward of 0.9521 and MSE of 0.0011 in this set-point tracking task. **Conclusion:** Our results show the feasibility of designing adaptive learning VNS control systems for regulating cardiovascular function using RL techniques.

Disclosures: P. Sarikhani: None. **H. Hsu:** None. **M. Zeydabadinezhad:** None. **Y. Yao:** None. **M. Kothare:** None. **B. Mahmoudi:** None.

Poster

P961. Experimental Computational Tools: SPARC

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P961.10

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

Support: NIH/SPARC 1 OT2 OD026585 01
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Customized SPARC transmission electron microscopy (TEM) tools for mapping of the autonomic nervous system and to guide new neuromodulation strategies

Authors: *N. P. BISCOLA[1], P. M. BARTMEYER[1], N. ZHANG[3], L. A. HAVTON[2,4]; [1]Neurol., [2]Neurol. and Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]David Geffen Sch. of Med. at UCLA, Los Angeles, CA; [4]James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: Detailed ultrastructural analysis and mapping of fibers and circuitry in the autonomic nervous system is important to identify new therapeutics for neuromodulation. We have developed customized transmission electron microscopy (TEM) analytical tools, including a correction factor approach to determine fiber size in oblique planes (Bartmeyer et al., Scientific Reports, 2021) , spatial distribution of fibers, and neuronal tracers and neurotransmitters by immunogold TEM. Nerve cross-sections do not provide images of perfectly rounded shapes of myelinated and unmyelinated axons. To better approximate and be closer to the real size of structures, we developed a mathematical approach based on the area and perimeter of an ellipse to determine morphometric parameters such as fiber diameter, axon diameter, myelin thickness, and g-ratio. The spatial distribution analysis provides additional descriptions of the nerve composition by considering the 2D position of each axon. Our statistical approach calculates the distance between the axon centroids and the center of the nerve and the distance between the axon centroids and the closest nerve epineurium. By crossing information from the initial dataset (area, diameter, myelin thickness, g-ratio of each axon) and the distance measurements, statistical tests verify if the axon population is evenly distributed or clustered within the nerve. Pre- and post-embedding immunogold detection techniques allows for the identification and characterization of neuronal tracers and neurotransmitters in the nervous system. Collectively, our integrated approach for TEM studies, with the introduction of our new analysis tools, allows for the identification of subsets of autonomic fibers with information relevant for the development of selective stimulation strategies. Furthermore, the implementation of standardized sampling techniques, for TEM data collection across multiple animal models and humans, and consideration of sexual dimorphism will promote increased rigor and reproducibility to guide new strategies for neuromodulation.

Disclosures: N.P. Biscola: None. **P.M. Bartmeyer:** None. **N. Zhang:** None. **L.A. Havton:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.01

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

Support: AFOSR Grant FA9550-16-1-0410
DARPA Contract HR0011-19-9-0035

Title: Untangling the Graph Structure of Drosophila Brain Datasets with Open Source FlyBrainLab Utility Libraries

Authors: A. A. LAZAR, M. K. TURKCAN, ***Y. ZHOU**;
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Abstract: We present a set of open source utility libraries written in Python to facilitate the exploration of the graph structure of fruit fly brain circuits from raw, large-scale connectomics and synaptomics datasets. The utility libraries are native in the interactive FlyBrainLab computing platform [1]. The main goal of these libraries is to extract the hidden circuits embedded in the connectome/synaptome, and to facilitate visualization of discovered circuit patterns. The utility libraries provide a large variety of tools, including algorithms for the discovery of connectivity clusters and patterns, acquiring morphometric measurements of neurons, 2D visualization of pathways as circuit diagrams or as 3D morphological views. These are described in more detail below.
The NeuroGraph library offers a set of tools to discover and analyze connectivity patterns between neuron groups of a given circuit. NeuroGraph provides an interface to connectomics datasets for the analysis of connectivity dendrograms, community detection algorithms, algorithms for investigating network controllability and comparison algorithms against random connectivity; in addition, it provides approaches to automatically generating 2D circuit diagrams. The NeuroEmbed library provides a group of algorithms for the discovery of graph embeddings into low-dimensional spaces. These feature representations provide a means for connectivity-driven cell type classification with and without morphometric features, a means to search for neurons similar to a given group of neurons with morphologically distinct but similar connectivity features. To standardize these capabilities, the library includes automated user-verifiable benchmarks for comparison of embedding algorithms on clustering and classification tasks.In addition, we provide three more libraries: (i) NeuroMetry that allows users to generate morphological measurements for neurons, (ii) NeuroSynapsis that facilitates retrieval and specification of circuits as well as statistical analysis of synaptomics data, and (iii) NeuroWatch

that provides additional utilities for 3D visualization of neurons and synapses in the FlybrainLab environment.
With examples focusing on multiple Drosophila neuropils including the antennal lobe, central complex and lateral horn, we reveal how our libraries can be used to uncover the functional logic of brain circuits. All libraries are available open source and can be found at https://github.com/FlyBrainLab/FBLClient.

References
[1] Lazar et al., eLife doi: 10.7554/eLife.62362, 2021.

Disclosures: A.A. Lazar: None. **M.K. Turkcan:** None. **Y. Zhou:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.02

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

Support: NIH Grant U24MH114827

Title: Multi-modal alignment of fMOST mouse brain imaging into the CCF through an intermediate atlas space

Authors: *M. CHEN[1], M. D. NAEEMI[2], Q. WANG[2], Y. WANG[2], S. A. SORENSEN[2], J. C. GEE[1], P. A. YUSKEVICH[1], L. NG[2];
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Abstract: Mesoscopic analysis of single neuron reconstruction from fluorescence micro-optical sectioning tomography (fMOST) is at the frontier of cell census studies of the mouse brain. fMOST imaging allows for sub-micron, 3D localization of individual neurons, enabling the study of cell morphology and connectivity in the whole brain. The Allen Common Coordinate Framework (CCF) [https://atlas.brain-map.org/] is a standard atlas space intended to allow mouse data from different specimens and modalities to be normalized, and then studied in tandem with over 400 manually annotated brain structures. However, accurate alignment of fMOST data to the CCF for group-level analysis remains an open challenge mainly due to modality differences and morphological changes during specimen preparation, which result in significant difficulties when establishing correspondences to analogous structures in the CCF. We introduce an approach for aligning individual fMOST images into the CCF using an intermediate, symmetric 25um isotropic fMOST atlas space constructed by applying the unbiased population-specific template algorithm (Joshi et al., 2004; Avants et al., 2004) to 60 fMOST images from 30 unique specimens. A detailed canonical coordinate transform is

established between this fMOST atlas and the CCF using 14 expert-generated annotations with matching correspondences in both atlases. Individual fMOST volumes are registered to the CCF in two phases: first the fMOST image is aligned to the fMOST atlas space using a standard deformable registration model, with minimal manual intervention. The resulting transformation is composed with the transformation between the fMOST and CCF atlases to establish the direct transformation between each fMOST image and the CCF. Preliminary analysis of our alignment shows that the large morphological expansions in the ventricles and between the cortex and the midbrain, characteristic of fMOST images, can be successfully collapsed into the corresponding structures in the CCF. Dice's coefficient was used to evaluate the overlap of the expert annotations after alignment. We found a high overlap of 0.98 for overall brain and ventricular morphology, and modest overlap for key structures, including the fimbria (0.62) and optic chiasm (0.77). Accurate alignment of individual fMOST data into the CCF establishes transformations that can be used to map single neuron reconstructions from different mice into the CCF for group level-spatial analysis. Our framework frontloads the manual intervention required for this alignment into a one-time atlas-to-atlas alignment while reducing individual fMOST alignments into simpler, mono-modality tasks.

Disclosures: M. Chen: None. **M.D. Naeemi:** None. **Q. Wang:** None. **Y. Wang:** None. **S.A. Sorensen:** None. **J.C. Gee:** None. **P.A. Yuskevich:** None. **L. Ng:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.03

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

Support: NSF Graduate Research Fellowship Program
NSF Grant CCF1839308

Title: Manifold learning to capture brain-state trajectories in fMRI

Authors: *E. L. BUSCH[1], J. HUANG[2], A. BENZ[3], T. WALLENSTEIN[2], G. LAJOIE[7,8], G. WOLF[7,8], S. KRISHNASWAMY[4,2,5], N. B. TURK-BROWNE[1,6];
[1]Psychology, [2]Computer Sci., [3]Mathematics, [4]Genet., [5]Program in Applied Mathematics, [6]Wu Tsai Inst., Yale Univ., New Haven, CT; [7]Mathematics & Statistics, Univ. de Montréal, Montreal, QC, Canada; [8]Mila - Quebec AI Inst., Montreal, QC, Canada

Abstract: Brain activity as measured with fMRI gives the illusion of intractably high dimensionality: densely-sampled data, rife with biological and collection noise. However, the intrinsic dimensionality of brain activity is far lower than its measurement dimensionality due to informational redundancy of individual voxels within and across regions and networks.

Accordingly, dimensionality reduction has yielded key insights into the neural dynamics of individuals and groups. Common approaches rely upon rudimentary linear algorithms, which neglect nonlinear interactions among the neural signals underlying fMRI activity. Here we use manifold learning to project fMRI data into a lower-dimensional space, where the dimensions of the space represent dominant functional activation patterns within a region of interest. We compare manifolds learned with PHATE, a cutting-edge diffusion-based algorithm designed to preserve both local and global signal structures, to conventional dimensionality reduction methods. In two fMRI datasets collected during naturalistic viewing, PHATE models reveal trajectories through brain states that reflect dynamic representations of complex stimuli. These trajectories correspond with aspects of the task that vary over the course of the experiment better than fMRI activity modeled in linear dimensions, indicating that dominant signals measured with fMRI encoding dynamic information arise from nonlinear interactions among neural populations. We probe this hypothesis further by adding a second affinity measure to PHATE to explicitly model the transitional probability between pairs of brain states, which we term *transitional PHATE* or T-PHATE. We evaluate the quality of manifold representations by quantifying the correspondence between each subject's fMRI timeseries within the manifold and aspects of the task they performed, including narrative trajectories, event segments, and visual stimuli. Manifolds learned by T-PHATE reveal trajectories through brain states which, when projected into as few as 3 dimensions, robustly represent local stimulus content and global narrative arcs. Across datasets and classification metrics, T-PHATE improves stimulus decoding accuracy relative to other dimensionality reduction methods and voxel-resolution data. These findings indicate that nonlinear manifolds can better model fMRI data than linear manifolds or voxel-resolution data, and by utilizing nonlinear manifolds, we can overcome much of the informational redundancy and noise associated with fMRI. The characteristics of these manifolds could in turn inform theories about the nature and dynamics of human brain function.

Disclosures: E.L. Busch: None. **J. Huang:** None. **A. Benz:** None. **T. Wallenstein:** None. **G. Lajoie:** None. **G. Wolf:** None. **S. Krishnaswamy:** None. **N.B. Turk-Browne:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.04

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

Support: NIH Grant U24EB029005
NSF Grant 1935749
NSF Grant 1935771
NIH Grant R01EB023297

Title: Large scale modeling and data processing and software dissemination using the Neuroscience Gateway

Authors: *A. MAJUMDAR[1], S. SIVAGNANAM[2], K. YOSHIMOTO[3], N. T. CARNEVALE[4], M. KANDES[3], S. YEU[3];
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Abstract: The Neuroscience Gateway (NSG) enables neuroscientists to run large scale models and process experimental data using high performance computing (HPC) and high throughput computing (HTC) computing resources. Computational modeling of cells and networks has become an essential part of neuroscience research, and investigators are using models on HPC resources to address problems of ever increasing complexity, e.g. large scale network models and optimization or exploration of high dimensional parameter spaces. Similarly processing of experimental data (EEG, MRI, fMRI etc.) increasingly require compute power of HTC. NSG democratizes computational and data processing neuroscience research by providing open and free access to HPC and HTC resources located at academic supercomputer centers in the US. NSG lowers or eliminates the administrative and technical barriers that make it difficult for investigators to use HPC and HTC resources. NSG has been in operation since early 2013. It provides around 15 neuroscience modeling and data processing software on HPC and HTC resources. It currently has about 1300 registered users. NSG has recently been integrated with the Open Science Grid (OSG) suitable for distributed HTC. We have also demonstrated a preliminary capability of NSG job submission to AWS cloud resources where the NSG jobs use the "cloudbursting" features of supercomputers to send jobs to AWS resources. Recently added new features to NSG include (i) transfer of large data directly to NSG's backend storage (in addition to be able to upload small input files via the web interface), (ii) sharing of data with other NSG users and their collaborators, (iii) processing publicly shared data by multiple users etc. Another new NSG feature is addition of a software development platform where neuroscience software developers get direct access and which provides a neuroscience software stack. Neuroscientists can use this platform to develop, benchmark, and scale their software. Robust software can be made available in containerized or cloud image form for dissemination either via NSG or otherwise for the neuroscience community. We have added a software repository and a web front end which provides detail information about the software such that users can use the software on NSG or their computing resources of choice such as commercial cloud. NSG is increasingly used in workshops, training classes and classroom teaching. Since its inception, NSG has enabled over 250 publications, presentations and thesis work.

Disclosures: A. Majumdar: None. **S. Sivagnanam:** None. **K. Yoshimoto:** None. **N.T. Carnevale:** None. **M. Kandes:** None. **S. Yeu:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.05

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

Support: NIDA 4R00DA045662-02 (SAG)
NIDA P30 DA048736 (SRON and SAG))
NARSAD Young Investigator Award 27082 (SAG)
NIDA T32 5T32NS099578-04 (NLG)
NIH F31 1F31MH125587-01 (NLG)

Title: Accessible and explainable machine learning for behavioral neuroscientists

Authors: *J. CHOONG, S. NILSSON, N. GOODWIN, V. TSAI, S. GOLDEN;
Univ. of Washington, Seattle, WA

Abstract: Background. Computational neuroethology has become an important component of modern behavioral neuroscience that is still challenging to implement for many research labs. We recently released an open-source package called Simple Behavioral Analysis (SimBA), which provides behavioral classification and analysis based on pose-estimation tracking for the greater behavioral neuroscience community. The goal of SimBA is to decrease the threshold to adopt machine learning-based methods while increasing the transparency and explainability of machine-learning derived results. **Method.** With the most recent release (github.com/sgoldenlab/simba), SimBA supports the following main applications in an inclusive and intuitive graphic interface: (*i*) generation of standard descriptive statistics and region-of-interest analysis for many open-source post-estimation architectures, (*ii*) generation and use of supervised behavioral classifiers, (*iii*) streamlined unsupervised analysis of supervised behavioral epochs, (*iv*) enhanced explainability of both supervised and unsupervised models using SHapley Additive exPlanations (SHAP), and *(v)* real-time behavioral classification for closed-loop applications. SimBA is based on a modular design philosophy so that other open-source applications can easily be integrated for either SimBA data import or compatible data export. Extensive documentation is provided at simba-docs.readthedocs.io. **Results.** SimBA enables accessible discovery of multi-relational and sequential behavioral patterns in single or multi-animal environments through decision ensembles and popular dimensionality reduction and clustering techniques. SimBA includes a range of visualization tools and dashboards for explainable classifications and real-time interactive data plotting and analysis. **Conclusion.** Although nascent, computational neuroethological approaches are now more accessible for behavioral neuroscientists. An inclusive open-source community is currently growing to support these approaches and SimBA provides a dynamic development platform within these efforts.

Disclosures: J. Choong: None. **S. Nilsson:** None. **N. Goodwin:** None. **V. Tsai:** None. **S. Golden:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.06

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

Title: Toward Quantitative Modeling of Variations in Autism Spectrum Disorder from Movement Data

Authors: *A. ASSADI[1], B. G. TRAVERS[2], **A. ARDALAN**[3];
[2]Waisman Center, Occup. Therapy Program in Kinesiology, [1]Univ. of Wisconsin - Madison, Madison, WI; [3]Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY

Abstract: Motivation - Prior work, including ours, has shown remarkable variation in the kinematics and balance outcomes for children with autism spectrum disorder (ASD), as well as significant variation in severity of ASD as measured by standardized test scores. Also, brain imaging indicates notable inter-individual variations. However, there is a paucity of quantifiable theoretical frameworks that account for the variations in brain and behavioral data, or explain the correspondence of the variations in these domains.
Thus, we develop a data-driven theoretical framework to organize variational parameters and ASD features. Researchers could use such a framework for articulating experimentally verifiable hypotheses, e.g. regarding the neural underpinnings of the behavioral features and their variations. We discuss the consequences of such a theoretical framework for organization of information from brain to behavior and their associations at multiple scales and resolutions.
Objectives - (1) Introduce a space-like quantifiable structure Q that captures variation in motor skills, social skills and cognitive flexibility from existing data, and allows incorporating future data in Q; (2) mathematically analyze Q and design algorithms for given data to be represented in Q.
Methods - (1) study movement data obtained from children with ASD while performing static Yoga and Tai Chi poses. We introduce a template T whose spatio-temporal changes captures the dynamics of human body movement, according to Gestalt principles and psychophysics. The dynamics of T corresponds to a physics-based simplification of the body movement dynamics. The variation in T yields a (moduli) space M which is the first approximation to Q. (2) utilize algebraic and differential geometry to construct auxiliary mathematical structures to enrich M as needed for inference about ASD variations from points, paths or other substructures afforded by M.
Results - Our framework provides a means for organizing historical experimental data (e.g. neuronal recording and imaging data) and localizing new experiments in M. This is possible by the existence of the push-forward metrics and moduli structures from Q to M. The correspondence between variation in motor performance and variation in neuronal dynamics makes it possible to unambiguously construct, among other artifacts, regression and classification models.

Disclosures: A. Assadi: None. **B.G. Travers:** None. **A. Ardalan:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.07

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

Support: NIH R01DC016621
NIH R03DC013990

Title: Uncued Speech Decoding with MEG

Authors: *D. DASH[1], P. FERRARI[2], J. WANG[1];
[1]Univ. of Texas at Austin, Austin, TX; [2]Helen DeVos Children's Hosp., Grand Rapids, MI

Abstract: Uncued Speech Decoding with MEG
Debadatta Dash[1], Paul Ferrari[1,2], Jun Wang[11] University of Texas at Austin, Austin TX, USA[2] Helen DeVos Children's Hospital, Grand Rapids, MI, USA
Neural speech decoding paradigms aim to decoding speech directly from the brain at natural communication rates that can assist patients with locked-in syndrome (e.g. due to amyotrophic lateral sclerosis, ALS). This contrasts with current commercial brain-computer interface spellers that can be slow (10 words/minute). However, most speech decoding studies are based on time-locked cued speech stimuli that may be decoding task effects rather than the speech-motor activation. For example, yes/no stimuli are very common in speech decoding studies but whether what's being decoded is the speech activity or the positive/negative brain state information associated with the task context, is not well known. To address this challenge, we collected magnetoencephalography (MEG) data in 7 subjects as they randomly spoke yes or no (~80 trials per word) at a self-paced rate in the absence of any cue, and present our analysis strategy for decoding speech information directly from the MEG signals. MEG has proven effective in tracking and decoding speech information in real-time due to its excellent temporal resolution. We extracted root mean square features form the MEG trials and trained a linear discriminant analysis (LDA) classifier with 4-fold cross-validation on train data to predict the labels (yes/no) from test data for each subject. Experimental results indicated that it is possible to decode uncued speech (yes/no) from the MEG signals with 79.02% ± 3.75% accuracy significantly higher than the chance level (1-tail t-test, $p<0.05$). The training cross validation accuracy was 83.09% ± 4.52%. This study demonstrates the efficacy of using MEG for uncued speech decoding and provides evidence for decoding speech activity rather contextual information.

Disclosures: D. Dash: None. **P. Ferrari:** None. **J. Wang:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.08

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

Support: European Research Council Grant 677697
NIH (1RF1-MH-123195-01)
(Interdisciplinary Grant ARUKIRG2019A-003
e EPSRC (EP-L016478-1, EP-M020533-1, EP-R014019- 1)
NIHR UCLH Biomedical Research Centre, the BRAIN Initiative Cell Census Network (U01-MH117023)
National Institute for Biomedical Imaging and Bioengineering (P41-EB-015896, 1R01-EB-023281, R01-EB006758, R21-EB-018907, R01-EB-019956, P41-EB-015902)
National Institute on Aging (1R56-AG064027, 1R01- AG064027, 5R01-AG008122, R01-AG016495)

Title: Synthesis of High-Resolution Research-Quality MRI Data from Low-Resolution Clinical MRI Data in Patients with COVID-19

Authors: *R. CALI[1], H. J. FREEMAN[1], B. BILLOT[4], D. B. FISCHER[5], W. SANDERS[1], M. BARRA[1], S. Y. HUANG[2], J. CONKLIN[3], B. FISCHL[3], J. IGLESIAS GONZALEZ[3], B. L. EDLOW[1];
[1]Neurol., [2]Neuroradiology, [3]Radiology, Massachusetts Gen. Hosp., Boston, MA; [4]Imperial Col. London, London, United Kingdom; [5]Harvard Med. Sch., Boston, MA

Abstract: Background/motivation: Neurological disorders are common in patients with coronavirus disease 2019 (COVID-19), but their underlying mechanisms are poorly understood. While hospitals worldwide are acquiring clinical MRI data in patients with COVID-19, these data lack sufficient resolution to perform cortical volumetric analyses that could provide critical insights into pathophysiologic mechanisms. Here, we apply a method for synthesis of high-resolution research-quality data from low-resolution clinical MRI data acquired in patients with COVID-19. We aimed to determine if synthesized images can be reliably used for cortical and subcortical volumetric analyses in these patients. Methods: We developed a joint super-resolution and synthesis method for generating 1 mm isotropic T1-weighted MPRAGE data from clinical MRI data of different orientation, resolution, and contrast. This method involves training a convolutional neural network (CNN) with highly diverse synthetic images generated from segmentations, which makes this technique (termed SynthSR) robust to changes in MR contrast, resolution and orientation of the clinical scans. We applied SynthSR to a dataset of nine critically

ill patients with severe COVID-19 scanned at our institution with both low-resolution clinical sequences, and a high-resolution, 1 mm isotropic T1-weighted MPRAGE sequence. We then synthesized images from the low-resolution data, and performed FreeSurfer-based cortical and subcortical volumetric analyses on both the synthesized and acquired MPRAGE. To measure the reliability of the synthesized data, we analyzed correlations between the volumetric measurements derived from each dataset.
Results: SynthSR successfully generated synthesized MPRAGE data for all nine patients. Correlations computed on cortical and subcortical volumetric measures were highest for global measures, including the cerebral cortex volume (r = 0.97), cerebral white matter volume (r = 0.92), and subcortical gray matter volume (r = 0.98), and lowest for the cerebellar white matter (r = 0.05 to 0.19). Conclusions: Synthesis of high-resolution images from low-resolution clinical MRI data creates an opportunity to examine thousands of clinical MRI scans acquired in patients with COVID-19 worldwide - scans that otherwise would not have sufficient resolution for such analysis. The SynthSR tool, which we distribute to the academic community at github.com/BBillot/SynthSR, can be used to support international collaboration in the study of COVID-19-related neurological disorders.

Disclosures: R. Cali: None. **H.J. Freeman:** None. **B. Billot:** None. **D.B. Fischer:** None. **W. Sanders:** None. **M. Barra:** None. **S.Y. Huang:** None. **J. Conklin:** None. **B. Fischl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CorticoMetrics. **J. Iglesias Gonzalez:** None. **B.L. Edlow:** None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.09

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

Title: Function and structure in cortical networks: using null models to interpret large-scale neural recordings

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Abstract: Modern recording technology enables the collection of vast quantities of neuronal data, permitting an unprecedented window on brain activity and the structural underpinnings of the circuits that generate this activity. Despite this volume of data, the number of parameters that would have to be specified to account for detailed structure-function relationships in cortical microcircuitry may be forever out of reach - and likely uninterpretable either way - necessitating alternative approaches to generate testable predictions for large-scale recordings across various

circuit structures, brain areas, individuals, and species. Moreover, determining which features of neural population activity dynamics (e.g., the structure of correlations in ensembles of cells; fluctuations in spiking rates; local field potential features) are generic features of a wide variety of network structures, and which are specifically tuned to particular systems, is a non-trivial task. Drawing on a series of recent papers [1 - 3], I suggest a roadmap for untangling the thorny relationship between structure and function across diverse neural recordings from a variety of species. Building a framework for extracting salient features of ensemble population dynamics that can be flexibly applied, I show how complex, multi-time scale dynamics can be identified across systems, and I connect these specific features in neuronal data to simple generative models. Thus, the interpretation of large-scale neural recordings requires the development of rigorous null models, with a natural starting point from (relatively) simple random-network models, with the aim of improving our ability to predict neural dynamics from structural information and vice versa. [1] Sederberg, Pala, Zhang, He, and Stanley, *PLoS-Computational Biology*, 2019; [2] Sederberg and Nemenman, *PLoS-Computational Biology*, 2020; [3] Morrell, Sederberg and Nemenman, *Physical Review Letters*, 2021

Disclosures: A. Sederberg: None.

Poster

P965. Computational and Analysis Tools IV

Location: Virtual Only

Time: Wednesday, November 10, 2021, 3:30 PM - 4:30 PM

Program #/Poster #: P965.10

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

Title: A personalized cortical atlas, generated from individual subject voxelwise connectivity

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Abstract: Functional connectivity, or the coactivations of resting state fMRI signal, varies by individual and has proven useful in describing brain organization. This macroscale organization has been described by a myriad of parcellation approaches based on various algorithms, modalities, and either group or individual approaches. However, the ‘best’ approach is unclear—the resolution and need for individualized methods likely vary depending on the research question. Here, we describe a parcellation method that integrates group and individual data and considers a range of resolutions using a large cohort from the Human Connectome Project (N = 1,018). We also describe solutions that approximate previous parcellations and identify resting state parcels that correspond with individual activity during tasks. First, we used the average high-resolution connectome from a subset of 500 individuals to find group-level parcellations using k-means clustering along a sequence of networks from 2 to 200. We found that the group-level solutions closely resembled major networks from previous group-level solutions reported in

Yeo et al., 2011. Second, we describe an unsupervised learning approach to calculate individualized parcellations based on a single subject's high-resolution connectome (from the remaining 518 individuals). Crucially, these individualized solutions are constrained by the group-level solutions and can thus be compared on a group-level while accounting for individual differences. Third, we identified group-level resting state parcels that closely matched individual task-based fMRI activity in motor, working memory, language, and high-level vision localizers. Individual task activity was defined using group-constraint subject-specific fROIs (Fedorenko et al., 2010), and we calculated the overlap between individual task activity and all previously identified individual resting state clusters. The motor-matched resting state parcels overall had the highest overlap with individual activity across subjects. These parcels were identified using the group of 500 individuals and replicated using the group of 518 individuals. Overall, we found that this parcellation methodology replicates previous group-level results, captures individual differences in functional connectivity at rest, and resembles an individual's task-based activity, particularly with motor localizers. Future work is necessary to decrease the computational load of these analyses, and to test the generalizability of these parcellations to other data and different cognitive tasks.

Disclosures: M. Molloy: None. **D.E. Osher:** None.